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Angiopoietin-like 4 serum levels on admission for acute myocardial infarction are associated with no-reflow

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ABSTRACT

Background: No-reflow in ST-segment elevation acute myocardial infarction (STEMI) is associated with a poor clinical prognosis. Its pathophysiological mechanisms are not fully elucidated yet but enhanced vascular permeability plays a key role in this phenomenon. Angiopoietin-like 4 (ANGPTL4) has been implicated in vascular permeability in experimental models of acute myocardial infarction (AMI). We therefore sought to investigate whether baseline ANGPTL4 serum levels are associated with no-reflow after primary percutaneous coronary intervention (PPCI).

Methods: We studied a group of 41 patients presenting with a first STEMI within 12 h of onset of symptoms and who underwent successful PPCI. Blood samples were obtained from all patients on admission before the start of the procedure, for ANGPTL4 level measurement. No-reflow was assessed by cardiac magnetic resonance imaging (MRI), the reference method.

Results: MRI-detected no-reflow was observed in 20 patients (48.8%). Variables independently associated with no-reflow on multivariate logistic regression analysis were: lower ANGPTL4 serum levels (odds ratio 0.82, 95% CI 0.70–0.98, $P = 0.02$), higher troponin T peak (odds ratio 1.03, 95% CI 1.00–1.05, $P = 0.03$), higher incidence of left anterior descending coronary artery (LAD) as culprit artery (odds ratio 14.61, 95% CI 1.24–172.49, $P = 0.03$), and higher C-reactive protein levels (odds ratio 1.18, 95% CI 1.00–1.39, $P = 0.05$).

Conclusion: ANGPTL4 serum levels predict MRI-detected no-reflow after successful PPCI in STEMI patients. Given the recently demonstrated therapeutic role of ANGPTL4 in diminishing no-reflow and limiting infarct size in preclinical animal models, these findings in humans may open up new possibilities in the field of research.

1. Introduction

Timely reperfusion by primary percutaneous coronary intervention (PPCI) is currently the most effective strategy of reperfusion in ST-segment elevation acute myocardial infarction (STEMI) [1–3]. Yet, successful restoration of epicardial coronary artery patency, as assessed by post-PCI angiography, does not necessarily prevent microvascular damage resulting from ischemia–reperfusion. In a variable proportion of patients presenting with STEMI, ranging from 5% to 50%, percutaneous coronary intervention (PCI) achieves epicardial coronary artery reperfusion but not myocardial reperfusion, a condition known as microvascular no-reflow [4–8]. The no-reflow phenomenon is a multifactorial process including tissue edema, endothelial cell swelling, vasoconstriction, inflammation and distal embolization of atheromatous and thrombotic debris. Vascular permeability plays a key role in this process in which ischemic and reperfusion injuries lead to destabilized leaky vessels and promote edema and leukocyte infiltration [9,10]. The no-

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reflow condition is a predictive factor of poor outcome with larger infarct size, increased incidence of heart failure and mortality [8,11,12]. Searching for new strategies that aim to improve acute myocardial reperfusion of acute myocardial infarction (AMI) has become incredibly challenging and preventing vascular permeability, which contributes to vascular damage, hemorrhages, edema and inflammation, is a recent avenue of research with new biological targets.

VEGF (vascular endothelial growth factor) also known as VPF (vascular permeability factor) is up-regulated during AMI [13]. Whereas its proangiogenic potential has been suggested in late phases of AMI, VEGF is also produced during the acute phase [14] and contributes to coronary vessel destabilization through activation of the Src Signaling pathway [15]. Since vascular damage is a critical component of the no-reflow phenomenon, blocking the VEGF-mediated leak at the acute phase of AMI might have a significant impact on limiting the extent of tissue injury [16].

Angiopoietin-like 4 (ANGPTL4) is a 55 kDa secreted protein induced by hypoxia [17] and *angptl4* mRNA is expressed in humans after AMI both in cardiomyocytes and endothelial cells [18]. We recently showed that ANGPTL4 inhibits VEGF-induced vascular permeability thus preserving endothelial cell barrier integrity in animal models of myocardial infarction (MI) [18]. Indeed, MI size was increased and major loss of integrity of coronary vessels was shown in *angptl4* knockout mice. The knockout phenotype was rescued by human recombinant ANGPTL4 administration, which counteracted ischemia-induced vascular endothelial growth factor signaling and disruption of endothelial cell junctions, thereby leading to subsequent protection of the coronary capillary network and reduction of the no-reflow phenomenon and infarct size [18].

However, the relationship between ANGPTL4 and the no-reflow phenomenon in humans has not been studied yet. In this study, we investigated whether ANGPTL4 serum levels on admission were associated with the occurrence of myocardial no-reflow in AMI patients treated by PPCI. No-reflow was assessed using myocardial magnetic resonance imaging (MRI), the reference method [19,20].

2. Methods

2.1. Population

We recruited 62 patients who presented as emergency cases at Pitié-Salpêtrière hospital with an acute STEMI. STEMI was defined by at least 2 of the 3 following conditions: at least 30 min of continuous chest pain, ST elevation > 0.2 mV in 2 or more adjacent electrocardiographic (ECG) leads, and further confirmation of necrosis by a rise of creatinine kinase (CK) level and troponin level to >twice the upper limit of normal.

Final inclusion criteria were the following: i) the current event was the first manifestation of coronary artery disease; ii) patients underwent PPCI within 12 h of symptom onset; iii) patients needed to have an occlusion of the culprit coronary artery [Thrombolysis in Myocardial Infarction (TIMI) flow 0 or 1] on the initial coronary angiogram and a successful PPCI as assessed by a final angiographic TIMI 3 flow; iv) MRI performed with good quality images.

The exclusion criteria included contraindications to MRI such as renal insufficiency (creatinine clearance < 30 ml/min/1.73 m^2), claustrophobia or material (e.g. pace-maker/defibrillator) contraindicating MRI, or cardiogenic shock at admission.

Of the 62 patients, 21 were excluded from the study, because of TIMI flow > 1 in the culprit coronary artery on admission (n = 10), post-PCI angiographic TIMI flow ≤ 2 (n = 4), contraindication to MRI (n = 5, of them, claustrophobia in 3, pace-maker in 1, refusal in 1) or insufficient quality of images for analysis (n = 2).

Thus a final group of 41 patients were included in the study. The study was approved by the Ethics Committee and informed consent was obtained from all the study subjects to use the blood samples for scientific purposes (e-paris registry).

2.2. PPCI procedure

All patients were treated with aspirin and clopidogrel. All PPCI procedures were performed via a radial approach. After conventional wire crossing and eventually thrombus aspiration, direct stenting was performed whenever possible, preceded by balloon pre-dilatation only if necessary. The use of GPIIbIIIa inhibitors, and of a device for thrombus aspiration was left to the operator's discretion. Coronary flow in the infarct-related artery before and after revascularization was graded according to the TIMI study group classification [21].

2.3. Laboratory assays

Peripheral blood samples for ANGPTL4 assessment were drawn in all patients on admission after vascular puncture, prior to heparin and GPIIbIIIa inhibitor administration. Blood was collected in tubes without any anticoagulant and centrifuged. Serum aliquots were stored at −80 °C in appropriate cuvettes until assayed. C-reactive protein serum levels were measured using an immuno-nephelometric high-sensitivity method. Serum levels of cardiac enzymes [phosphocreatine kinases M and B (CK-MB) and troponin T] were measured, according to hospital protocol, every 6 h during the first day and every 12 h in the following 3 days using standardized methods. Serum VEGF levels were measured by radio-immunoassay using a commercial kit (Human VEGF ELISA Kit; Biosource/Invitrogen). Serum ANGPTL4 levels were measured by a home-made ELISA assay using monoclonal anti-ANGPTL4 antibodies which have already been described [22]. The sandwich ELISA protocol is detailed in Supplementary material and methods. In all patients, left ventricular ejection fraction (LVEF) was measured in the coronary care unit after the PPCI procedure using 2D echocardiography (Simpson method).

2.4. Cardiac magnetic resonance (CMR) imaging

2.4.1. CMR protocol

MRI represents the reference method to identify no-reflow and directly quantify infarct size [19,20,23]. Infarct size and no-reflow were thus determined by MRI only.

All CMR studies were performed on Philips Achieva 1.5 Tesla MR systems (Philips Healthcare, Best, The Netherlands), using the same scanning protocol. Cine images of the entire left ventricle were acquired using an ECG-gated balanced steady state free precession (SSFP) pulse sequence. Following this, an intravenous bolus dose of 0.1 mmol/kg Gd-DOTA (DOTAREM, Guerbet, France) was administered at a rate of 3 ml/s by a power injector (Medrad Spectris Solaris, Medrad, USA). First-pass perfusion imaging was performed simultaneously with the injection of gadolinium. Data from these two sequences were not used for data analysis.

Immediately after first-pass perfusion imaging, a second bolus dose of 0.1 mmol/kg Gd-DOTA was administered. Ten minutes after gadolinium injection, a 'Look Locker' sequence was performed to obtain the most appropriate fixed inversion time (TI) to null the signal intensity of normal myocardium. The minimum TI for the study group was 225 ms and the maximum was 250 ms. The median TI was 240 ms. Late gadolinium enhancement (LGE) images were then acquired during diastole 16.8 + −5.6 min after gadolinium injection with the following pulse sequence parameters: inversion recovery-prepared T1-weighted gradient echo, TR 4.9 ms, TE 1.9 ms, flip angle 15°, spatial resolution 1.35 mm × 1.35 mm × 10 mm, field of view (FOV) range 350–400 mm, and one three-dimensional slice covering the entire left ventricle (LV) in short axis.