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Release of endothelial microparticles in patients with arterial hypertension, hypertensive emergencies and catheter-related injury

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ABSTRACT

Background and aims: Circulating endothelial microparticles (EMPs) are increased in arterial hypertension. The role of physicomechanical factors that may induce EMP release *in vivo* is still unknown. We studied the relationship of EMPs and physicomechanical factors in stable arterial hypertension and hypertensive emergencies, and investigated the pattern of EMP release after mechanical endothelial injury.

Methods: In a pilot study, 41 subjects (50% hypertensives) were recruited. EMPs were discriminated by flow-cytometry ($CD31^+/41^-$, $CD62e^+$, $CD144^+$). Besides blood pressure measurements, pulse-wave-analysis was performed. Flow-mediated dilation (FMD), nitroglycerin-mediated dilation (NMD), and wall-shear-stress (WSS) were measured ultrasonographically in the brachial artery; microvascular perfusion by laser-Doppler (Clinicaltrials.gov: NCT02795377). We studied patients with hypertensive emergencies before and 4 h after BP lowering by urapidil ($n = 12$) and studied the release of EMPs due to mechanical endothelial injury after coronary angiography ($n = 10$).

Results: Hypertensives exhibited increased EMPs ($CD31^+/41^-$, $CD144^+$, $CD62e^+$) as compared to normotensives and EMPs univariately correlated with systolic BP (SBP), augmentation index, and pulse wave velocity and inversely with FMD. $CD31^+/41^-$-EMPs correlated with diameter and inversely with WSS and NMD. $CD62e^+$ and $CD144^+$-EMPs inversely correlated with microvascular function. During hypertensive emergency, only $CD62e^+$ and $CD144^+$-EMPs were further elevated and FMD was decreased compared to stable hypertensives. Blood pressure lowering decreased $CD62e^+$ and $CD144^+$-EMPs and increased FMD. $CD31^+/41^-$EMPs, diameter, and WSS remained unaffected. Similar to hypertensive emergency, catheter-related endothelial injury increased only $CD144^+$ and $CD62e^+$-EMPs.

Conclusions: EMP release in hypertension is complex and may involve both physicomechanical endothelial injury and activation ($CD144^+$, $CD62e^+$) and decreased wall shear stress ($CD31^+/41^-$).

1. Introduction

Arterial hypertension is an important risk factor facilitating the development and progression of atherosclerosis. While arterial hypertension is associated with increased mechanical stress on the arteries leading to macrovascular stiffening and microvascular dysfunction, it is not entirely clear how it is linked to atherosclerosis progression [1]. Stiffness of conduit arteries leads to accelerated PWV, which in turn enhances transmission of mechanical forces to the microcirculation. While the larger arteries are exposed to higher pulsatility leading to facture of load bearing wall components and positive remodeling with increased diameters and stiffness, the pulse reflection goes along with force transmission that is associated with microvascular remodeling, rarefaction, and microvascular dysfunction. It could be hypothesized that the mechanical stress may lead to endothelial injury and dysfunction

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which, in turn, could drive arterial remodeling and plaque development [2,3].

According to the response to injury theory, mechanical injury and exposure to cardiovascular risk factors disrupt endothelial integrity driving the initiation and progression of atherosclerosis [4]. Endothelial microparticles (EMPs) can be viewed as circulating markers of endothelial injury and a compromised endothelial integrity that are released from activated and apoptotic endothelial cells as shown *in vitro* [5,6]. These EMPs are membrane particles of less than a micrometer in diameter and carry endothelial surface markers [5–7] and enzymes including eNOS [8]. Circulating levels of EMPs increase in plasma early in the atherosclerotic processes, correlate with the degree of endothelial dysfunction [8,9], and have been established as prognostic biomarkers that predict adverse CV outcome [10–12]. Cardiovascular risk factors may trigger EMP release [7,13]. While it was previously shown that arterial hypertension goes along with increases EMP levels in human subjects [14–16], the role of physicomechanical factors present in arterial hypertension that may induce mechanical endothelial injury and activation and shedding of EMPs *in vivo* is still unknown.

In order to investigate this, we first studied in a cross-sectional pilot cohort how hemodynamic, structural, and functional characteristics that are chronically changed in stable hypertensives relate to circulating EMP concentrations. We then evaluated the relationships of arterial characteristics and the pattern of EMP dynamics during hypertensive emergencies and after consecutive blood pressure lowering. Finally, we determined the pattern of EMPs released by a prototypical mechanical endothelial injury as induced during arterial catheterization.

2. Materials and methods

2.1. Study subjects and protocol

In a first pilot study (study 1), we investigated circulating EMPs along with functional and mechanical characteristics of the arterial system in 41 consecutive male subjects, 20 subjects without arterial hypertension and 21 subjects with arterial hypertension as defined by office blood pressure ≥140/90 mmHg [17] or history of hypertension with ongoing antihypertensive medication (Table 1). The patients were recruited from the out-patient clinic. As the presence of CAD might also affect circulating EMP values, we aimed at including 50% patients with stable CAD. No dedicated matching procedures were applied. Hemodynamics and endothelial function of the brachial artery were assessed as flow-mediated dilatation (FMD). Pulse wave analyses including central blood pressure, pulse wave velocity (PWV), and aortic augmentation index (AIX) were performed by tonometry. In all subjects, EMP subpopulations ($CD31^+/41^-$, $CD144^+$, $CD62e^+$) were analyzed by flow-cytometry according to the expression of surface antigens. Exclusion criteria were manifest peripheral artery, or cerebrovascular disease, acute inflammation (C-reactive protein [CRP] >0.6 mg/dl), kidney failure (estimated glomerular filtration rate [eGFR] <30 ml/min), malignancies, and arrhythmias (heart rhythm other than sinus).

Table 1
Characteristics of study population (study 1).

	NT	HT	*p*-value
n	20	21	
CAD	10	10	
Diabetes mellitus	0	0	
Smoker	0	0	
Age (y)	60 ± 4.7	59 ± 6.0	0.619
BMI (kg/m^2)	27.1 ± 3.6	29.2 ± 3.7	0.081
Height (m)	1.8 ± 0.6	1.8 ± 0.6	0.636
Weight (kg)	86 ± 12	93 ± 12	0.081
Creatinine (mg/dl)	0.9 ± 0.1	1.0 ± 0.1	0.591
Urea (mg/dl)	35 ± 11	33 ± 8	0.584
Total cholesterol (mg/dl)	199 ± 43	191 ± 51	0.623
LDL cholesterol (mg/dl)	138 ± 31	140 ± 40	0.866
HDL cholesterol (mg/dl)	52 ± 15	55 ± 28	0.671
Triglycerides (mg/dl)	134 ± 62	139 ± 34	0.746
Fasting plasma glucose (mg/dl)	102 ± 20	99 ± 10	0.506
HbA_{1c} (%)	5.5 ± 0.5	5.5 ± 0.4	0.738
CRP (mg/dl)	0.1 ± 0.2	0.1 ± 0.2	0.578
Hb (mg/dl)	14.5 ± 1.2	14.8 ± 1.5	0.410
Leucocytes (1000/μl)	6.3 ± 1.3	7.4 ± 1.4	0.394

Values are mean and standard deviation; *p*-values refer to unpaired *t*-test.

In a second study, we analysed 12 patients that were admitted to the emergency department with acute symptoms of dyspnea or angina in the presence of blood pressure >180/120 mmHg, troponin T value not exceeding the upper reference limit (14 ng/l) at admission and 1 h excluding acute myocardial infarction and indicating hypertensive emergency [17]. Measurements of EMPs and vascular measurements were taken at admission (0 h) before and at 4 h and after lowering of BP by urapidil (Stragen, Denmark, 10–50 mg i.v.). EMP subpopulations were analyzed by flow-cytometry and FMD measured by ultrasound. All patients were discharged on the same day. Exclusion criteria were manifest peripheral artery, or cerebrovascular disease, acute inflammation (CRP>0.6 mg/dl), kidney failure (eGFR<30 ml/min), malignancies, arrhythmias (heart rhythm other than sinus), and troponin T value exceeding the upper reference limit (14 ng/l).

In a third series, we aimed at evaluating the pattern of EMP release due to a prototypical mechanical endothelial injury as exerted by a catheter moved through large arteries. Therefore, we studied EMPs release and vascular function before, at 1 h, at 4 h, and at 24 h after elective transfemoral diagnostic coronary angiography in n = 10 stable CAD in-patients without arterial hypertension. EMP subpopulations were analyzed by flow-cytometry and FMD measured by ultrasound. Inclusion criteria were normal left ventricular ejection fraction (>55%), stable CAD, and blood pressure <140/90 mmHg. Exclusion criteria were manifest peripheral artery, or cerebrovascular disease, acute inflammation (CRP>0.6 mg/dl), kidney failure (eGFR<30 ml/min), malignancies, and arrhythmias (heart rhythm other than sinus).

Measurements were always performed in the same order in one session. We first took blood samples from the left arm. After a 15 min supine resting period in a quite air conditioned room, we performed FMD measurements on the right arm, then laser Doppler measurements on the left arm, followed by blood pressure measurements on the right arm, and, finally, performed applanation tonometry on the neck and groin. The study protocol was approved by the ethics committee of the Heinrich Heine University Duesseldorf and all patients and volunteers gave written informed consent (Clinicaltrials.gov: NCT02795377).

2.2. Characterization of EMP subpopulations by flow cytometry

Citrated blood (6 ml) was drawn from the cubital vein and processed within 2 h. Platelet-rich plasma was obtained by centrifugation of whole blood at 300 × *g* over 15 min at room temperature. Platelet-free plasma was obtained by 2 successive centrifugations of platelet-rich plasma at 10,000 × *g* for 5 min at room temperature. Briefly, samples were incubated for 30 min with fluorochrome-labeled antibodies or matching isotype controls and analyzed in a Canto II flow cytometer (Beckton Dickinson, Heidelberg, Germany). Microbead standards (1.0 μm) were used to define MPs as <1 μm in diameter. The EMP subpopulations were defined as $CD31^+/CD41^-$, $CD62e^+$, or $CD144^+$ events. The total number of EMPs was quantified using flow-count calibrator beads (20 μl).