Thrombosis Research 170 (2018) 1–9

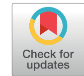
Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Full Length Article

Selective increase of cardiomyocyte derived extracellular vesicles after experimental myocardial infarction and functional effects on the endothelium☆



Jose A. Rodriguez[a,b,c], Josune Orbe[a,b,c], Goren Saenz-Pipaon[a], Gloria Abizanda[c,d], Natalia Gebara[a], Flavia Radulescu[a], Pedro M. Azcarate[e], Luis Alonso-Perez[e], David Merino[f], Felipe Prosper[d,d,g], Jose A. Paramo[a,b,c,d], Carmen Roncal[a,b,c,*]

[a] Laboratory of Atherothrombosis, Program of Cardiovascular Diseases, Center for Applied Medical Research (CIMA)-University of Navarra, Spain
[b] CIBERCV, Madrid, Spain
[c] IdiSNA, Instituto de Investigación Sanitaria de Navarra, Spain
[d] Hematology Service, Clínica Universidad de Navarra, University of Navarra, Pamplona, Spain
[e] Departamento de Cardiología, Hospital San Pedro, Logroño, Spain
[f] Flow Cytometry and Cell Sorting Core, Health Research Institute-IDIVAL, Santander, Spain
[g] CIBERONC, Madrid, Spain

ARTICLE INFO

Keywords:
Extracellular vesicles
Ischemia
Intercellular communication
Cardiac repair
Endothelium

ABSTRACT

Introduction: Wound healing after myocardial infarction (MI) is mediated by different cell types, secreted proteins, components of the extracellular matrix (ECM) and, as increasing evidences suggest, extracellular vesicles (EVs). We aim to determine the dynamics of release and origin of EVs after MI, as well as their biological activity on endothelial cells (ECs).
Methods: MI was induced in WT mice and blood and tissues collected at baseline, 3, 15 and 30 days post-ligation for cardiac function (echocardiography) and histological evaluation. Circulating EVs subpopulations were measured by flow cytometry in mouse, and in a small cohort of patients with ST-segment elevation MI (STEMI, $n = 6$). In vitro, EVs were isolated from a cardiomyocyte cell line (HL1) and their function assayed on ECs.
Results: Leukocyte and endothelial EVs increased concomitant to inflammatory and angiogenic processes triggered by ischemia. More strikingly, cardiomyocyte EVs (connexin43^{+}) were detected in STEMI patients and in murine MI, where a significant increase in their levels was reported at day 15 post-ischemia ($p < 0.05$ vs baseline). In vitro, HL1EVs induced ECs migration ($p = 0.05$) and proliferation ($p < 0.05$), but impaired tube formation. These apparent contradictory results could be partially explained by the upregulation of MMP3, and the apoptosis and senescence genes, *p53* and *p16*, induced by HL1EVs on ECs ($p < 0.05$).
Conclusions: MI induces the release of different EVs subpopulations, including those of cardiac origin, in a preclinical model of MI and STEMI patients. In vitro, cardiomyocyte derived EVs are able to modulate endothelial function, suggesting their active role in heart repair after ischemia.

1. Introduction

Following myocardial infarction (MI), inflammation, extracellular matrix (ECM) turnover, angiogenesis and cardiomyocyte hypertrophy are activated in a finely orchestrated process involving different cellular components [1,2]. Cell communication therefore, seems essential to

Abbreviations: CV, cardiovascular; ECs, endothelial cells; ECM, extracellular matrix; EVs, extracellular vesicles; FACs, flow cytometry; H&E, hematoxylin eosin; ICAM, intercellular adhesion molecule; LAD, left anterior descending; MMPs, matrix metalloproteinases; MV, microvesicles; MLECs, mouse lung ECs; MI, myocardial infarction; PFP, platelet-free plasma; RT-PCR, real time polymerase chain reaction; STEMI, ST-segment elevation myocardial infarction; VCAM, vascular cell adhesion molecule; WT, wild type

☆ Preliminary data of this work were presented at the National Congress of the Spanish Society of Thrombosis and Haemostasis (SETH) 2016 and at the International Society of Thrombosis and Haemostasis (ISTH) 2017 as a poster presentation.

* Corresponding author at: Laboratory of Atherothrombosis, Program of Cardiovascular Diseases, CIMA-University of Navarra, Avda. Pio XII, 55, 31008 Pamplona, Navarra, Spain.
E-mail address: croncalm@unav.es (C. Roncal).

https://doi.org/10.1016/j.thromres.2018.07.030
Received 4 June 2018; Received in revised form 13 July 2018; Accepted 31 July 2018
Available online 01 August 2018
0049-3848/ © 2018 Elsevier Ltd. All rights reserved.

modulate this response. In this regard, extracellular vesicles (EVs) have emerged as a potential mechanism for cell to cell interaction by shuttling bioactive molecules able to modulate extracellular environment and cell behaviour [3].

EVs are small vesicles that can be released from all cell types during diverse physiological and pathological processes, and are capable of transferring biological information to target cells. They express markers that indicate changes in apoptosis or activation state of their cells or organs of origin, and are considered as intercellular messengers, as well as biomarkers of cell injury or activation [4]. As no straightforward criteria exist to distinguish, isolate and identify vesicle sub-populations, the term EVs has been introduced as a common and collective name for exosomes and microvesicles (MVs) [5]. EVs are attracting considerable interest in the cardiovascular (CV) field as a wide range of their functions is recognized. Several studies report increased levels of EVs after MI and their ability to predict future risk of CV events in patients with coronary artery disease [6–10]. However, it is still controversial which EVs subpopulations (endothelial, platelet, etc.) are most useful for diagnostic or prognostic purposes [9,11–13]. Moreover, the dynamic of their release and clearance remains unclear. Besides their role as biomarkers, EVs also display biological activities and induce cellular responses in vitro and in vivo [4].

During MI, ischemia triggers the formation of new blood vessels to ensure tissue oxygenation after blood shortage. At the infarcted region angiogenesis is activated early post-MI (granulation phase) to quickly restore blood flow and prevent cardiomyocyte death [14]. The role of cardiomyocyte or cardiac stem cell-derived EVs in neovascularization has been studied in vitro and in vivo, showing both pro- and anti-angiogenic activities depending on the stimulus triggering their release [15–17]. Moreover, as messenger of intercellular communication, EVs could mediate a crosstalk between endothelial cells (ECs) and cardiomyocytes during tissue repair [18].

We used a mouse model of MI to study the dynamics of EVs release in vivo and whether EVs of cardiac origin modulated EC function in vitro. Our specific aims were to; 1) determine changes in EV number and cellular origin at different time points after MI in WT mice, 2) correlate the fluctuation in EVs with the morphological changes in the heart, and 3) assess in vitro the effect of cardiomyocyte derived EVs on endothelial function. In addition, to support the clinical relevance of this in vivo model, a small cohort of patients with ST-segment elevation MI (STEMI) was studied.

2. Materials and methods

Appendix A includes an expanded and detailed version of this section.

2.1. Myocardial infarction model, histological analysis and flow cytometry

Cardiac ischemia was induced in C57BL/6J adult mice (12-week-old, male, Harlan, $n = 5–6$/group) by permanent ligation of the left anterior descending (LAD) coronary artery as previously described [19]. Ultrasound images were acquired at baseline (before LAD ligation), and 2, 15 and 30 days after LAD ligation with a Vevo 770 ultrasound system (Visualsonics) [20].

Mice were euthanized at day 3, 15 and 30 post-ligation by CO_2 inhalation and perfused with saline. Then, hearts were dissected and tissues fixed overnight in 2% phosphate-buffered paraformaldehyde (PFA), dehydrated, and embedded in paraffin. Heart sections were stained with rat anti-mouse CD31 (Dianova), rat anti-mouse F4/80 (Bio-Rad), and rat anti-mouse NIMP-R14 (Abcam) antibodies followed by incubation with peroxidase-labelled secondary IgGs (Dako) and corresponding tyramide amplification systems (PerkinElmer). Hematoxylin eosin (H&E) and Sirius red stainings were used to evaluate infarct size and total collagen deposition. Morphometric analysis was performed using a Nikon Eclipse 80i microscope (Nikon Instruments) with Cellˆ D software for collagen and Fiji image analysis software in any other case.

50 μL of blood were collected at baseline and 24 h post-MI from the orbital sinus of anaesthetized mice into heparin (10 U/mL) treated capillaries. Circulating leucocytes were determined by FACs (FACSCalibur, BD Bioscience) with a PE rat anti-mouse CD11b antibody (BD-Biosciences) and their numbers normalized with calibrated beads (Perfect-Count Microspheres, Cytognos).

Experiments were performed in accordance with European Communities Council Directives (2010/63/EU) guidelines for the care and use of laboratory animals and were approved by the University of Navarra Animal Research Review Committee (Protocol number 055-14).

2.2. Characterization and quantification of mouse EVs in plasma

Blood was drawn from the heart of anaesthetized mice (starved for 6 h) into 0.0129 M citrate at baseline and 3, 15 and 30 days post-ischemia and spun-down twice at 1560 $\times g$ for 20 min at 20 °C to collect platelet-free plasma (PFP). PFP was snap frozen and stored at −80 °C. EVs were labelled with specific monoclonal antibodies: PE anti-CD62E for endothelium, PE anti-CD41 for platelets, PE rat anti-mouse CD11b for leukocytes, and APC TER119 for erythrocytes (all from BD-Biosciences). Cardiomyocyte derived EVs were detected with a rabbit anti-connexin-43 antibody (ProteinTech), labelled before staining with Zenon Alexa Fluor 488 rabbit IgG labelling Kit (Life technologies) following the manufacturer's instructions.

FACs was performed on BD FACSCanto II cytometer (BD Biosciences) 20 min after incubation with the corresponding antibodies. Isotype control antibodies IgG2a-PE, IgG1-PE, IgG2b-APC were used as negative controls. FlowJo analysis software version 9.3 was used to analyze the results.

EVs quantification was performed using 25 μL > flowcount beads (diameter 1.34 μm, Spherotec). The number of EVs was calculated using the following formula: $[EVs] / \mu L >= (NMP \times 1000) / NB$ where NEV = total EV counts, NB = total Bead counts and concentration for beads/μL > = 1000.

2.3. EVs isolation from HL1 cell culture and characterization

HL1 cardiac muscle cell line [21] derived EVs were isolated from cell culture supernatants by ultracentrifugation. Briefly, cells were grown to confluency in complete Claycomb Medium (Sigma) 10%FBS (Sigma), 0.1 mM norepinephrine (Sigma), 2 mM L-Glutamine (Gibco, ThermoFisher), 1% penicillin/streptomycin (P/S), and changed to serum free medium (Claycomb medium. 0.1 mM norepinephrine, 2 mM L-Glutamine, 1% P/S) for 24 h. The medium was changed again and cells were incubated for 24 h in serum free medium. Then, culture supernatants were collected and cleared from detached cells or large cell fragments by a double centrifugation at 300 $\times g$ for 5 min followed by one of 2500 $\times g$, 10 min. Then EVs were pelleted at 20,000 $\times g$, 70 min at 4 °C. Pelleted EVs were washed once in HEPES-NaCl buffer (10 mM Hepes, 0.9% NaCl, pH = 7.4) for 70 min at 20,000 $\times g$, at 4 °C. Finally, pelleted EVs were resuspended in HEPES-NaCl buffer and stored at −80 °C. Protein content was measured with Nanodrop (ThermoFisher).

5 μg of protein from lysed HL1EVs (3xLiqN_2-37 °C), were immunoblotted for Alix (mouse Anti-AIP1, BD Bioscience) and Calnexin (rabbit Anti-Calnexin, Abcam) antibodies. β-actin (Sigma) was used as loading control.

HL1EVs particle size distribution was measured using Nanoparticle Tracking Analysis (NANOSIGHT NS300) and Dynamic Light Scattering (Zetasizer Nano) following the manufacturer's instructions (Malvern Instruments Limited).

Download English Version:

https://daneshyari.com/en/article/8679258

Download Persian Version:

https://daneshyari.com/article/8679258

Daneshyari.com