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Particulate matter induces prothrombotic microparticle shedding by human mononuclear and endothelial cells

Tommaso Neri [a], Laura Pergoli [b], Silvia Petrini [a], Lotte Gravendonk [b], Cristina Balia [a], Valentina Scalise [a], Angela Amoruso [c], Roberto Pedrinelli [a], Pierluigi Paggiaro [a], Valentina Bollati [b,d], Alessandro Celi [a,*]

[a] Laboratory of Respiratory Cell Biology, Department of Surgical, Medical, Molecular Pathology and Critical Area and Azienda Ospedaliero-Universitaria Pisana, Università degli Studi di Pisa, Pisa, Italy
[b] EPIGET - Epidemiology, Epigenetics and Toxicology Lab, - Department of Clinical Sciences and Community Health, Università degli Studi di Milano, Milan, Italy
[c] Department of Pharmaceutical Sciences and Department of Medical Sciences, Università del Piemonte Orientale, Italy
[d] Epidemiology Unit, Fondazione Cà Granda, IRCCS Ospedale Maggiore Policlinico, Milan, Italy

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ABSTRACT

Particulate airborne pollution is associated with increased cardiopulmonary morbidity. Microparticles are extracellular vesicles shed by cells upon activation or apoptosis involved in physiological processes such as coagulation and inflammation, including airway inflammation. We investigated the hypothesis that particulate matter causes the shedding of microparticles by human mononuclear and endothelial cells.

Cells, isolated from the blood and the umbilical cords of normal donors, were cultured in the presence of particulate from a standard reference. Microparticles were assessed in the supernatant as phosphatidylserine concentration. Microparticle-associated tissue factor was assessed by an one-stage clotting assay. Nanosight technology was used to evaluate microparticle size distribution.

Particulate matter induces a dose- and time- dependent, rapid (1 h) increase in microparticle generation in both cells. These microparticles express functional tissue factor. Particulate matter increases intracellular calcium concentration and phospholipase C inhibition reduces microparticle generation. Nanosight analysis confirmed that upon exposure to particulate matter both cells express particles with a size range consistent with the definition of microparticles (50–1000 nm).

Exposure of mononuclear and endothelial cells to particulate matter upregulates the generation of microparticles at least partially mediated by calcium mobilization. This observation might provide a further link between airborne pollution and cardiopulmonary morbidity.

1. Introduction

Particulate matter (PM) is a complex mixture of small particles and liquid droplets. Particle pollution is made up of a number of components, including acids (such as nitrates and sulfates), organic chemicals, metals, and soil or dust particles. The size of particles is directly linked to their potential to cause health problems, as it is in turn directly linked to the ability of particles to penetrate more or less deeply into the respiratory system. PM exposure has wide effects on health (Alfaro-Moreno et al., 2007) and a large body of evidence has consistently shown that both short and long term exposures to PM are associated with increased cardiovascular and pulmonary related morbidity and mortality [see (Pelucchi et al., 2009) and (Franklin et al., 2015) for comprehensive reviews]. However, the mechanisms behind this association are not fully understood.

Microparticles (MP), also referred to as microvesicles or ectosomes, are small (diameter 0.05–1 μm) vesicles that originate from the cell surface of most (if not all) cell types during activation or apoptosis. MP are heterogeneous in nature, varying in both size and content, and present cell surface markers and cytoplasmic components of the parent cells from which they originate. MP are involved in numerous physiologically relevant processes, such as blood coagulation (Falati et al., 2003, Celi et al., 2004) and inflammation (Celi et al., 2004, Leroyer et al., 2010, Ardoin et al., 2007, Distler et al., 2006), including airway inflammation (Cerri et al., 2006, Neri et al., 2011, Cordazzo et al., 2014). Accordingly, MP have recently gained attention as both biomarkers and effectors in human diseases, including cardiac and pulmonary diseases (Takahashi et al., 2014, Takahashi and Kubo, 2014, Amabile et al., 2014, Hu et al., 2014, Duarte et al., 2013).

Abbreviations: PBS, phosphate buffered saline; TF, tissue factor; LPS, phosphatidylserine; MP, microparticles; PM, particulate matter; PBMC, peripheral blood mononuclear cells; HUVEC, human umbilical vein endothelial cells; SRM1648a, standard reference material 1648a Urban Particulate Matter.

* Corresponding author at: Dipartimento Cardiotoracovascolare, Ospedale di Cisanello, Via Paradisa, 2, – 56124 Pisa, Italy.
E-mail address: alessandro.celi@med.unipi.it (A. Celi).

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Two well-known cellular processes lead to the formation of MP, i.e., cell activation, either chemical or physical, by agonists or shear stress respectively, and apoptosis, through the action of growth factor deprivation or apoptotic inducers (VanWijk et al., 2003, Mostefai et al., 2008). We have previously demonstrated that a number of agonists, including two different peroxisome proliferator-activated receptor-γ agonists, angiotensin II and cigarette smoke extract cause a rapid, calcium dependent, upregulation of MP shedding from leukocytes and that different calcium inhibitors largely prevented MP shedding (Neri et al., 2012, Cordazzo et al., 2013, Cordazzo et al., 2014). Since PM induces cytosolic calcium mobilization in monocytes (Brown et al., 2007), we speculated that PM, more specifically PM10, induces the generation of MP by peripheral blood mononuclear cells (PBMC) and human umbilical vein endothelial cells (HUVEC), thus providing a novel link between airborne pollutants and cardiopulmonary risk.

2. Materials and methods

2.1. Reagents and kits

RPMI 1640 medium, penicillin, streptomycin, L-glutamine, fetal bovine serum, trypan blue, phosphate buffered saline, Ficoll-Histopaque, dextran T500 and U73122 were obtained from Sigma (Milano, Italy). Thromboplastin standard was obtained from Beckman Coulter (Milano, Italy). Human anti-tissue factor (TF) antibody was obtained from America Diagnostica (Instrumentation Laboratory, Milano, Italy). The Zymuphen MP-Activity kit was obtained from Hyphen BioMed (Neuville-sur-Oise, France). The Fluo-4 NW Calcium Assay kit was obtained from Molecular Probes (Invitrogen, Milano, Italy). Standard reference material 1648a Urban Particulate Matter (SRM1648a) was obtained from National Institute of Standard Technology (Gaithersburg, MD, USA).

2.2. Cell isolation and culture

2.2.1. HUVEC

HUVEC were isolated from umbilical vein cords as described (Del Fiorentino et al., 2010) by digestion with 0.1% collagenase (specific activity: 316 U/mL, Gibco, Invitrogen) and grown to confluence at 37 °C in 5% CO_2 humidified incubator, on 25-cm^2 tissue culture flasks previously coated with 1% gelatine in supplemented culture medium (M199 with 10% heat inactivated fetal calf serum, 100 U/mL penicillin, 100 μg/mL streptomycin, 2 mM L-glutamine, 10 mM HEPES pH 7.4, heparin 12 IU/mL, 1% retinal derived growth factor, all from Sigma). Following trypsin treatment, the cells were detached from the flasks and final monolayers were prepared by seeding HUVEC on gelatine-precoated culture plates and then incubated for 24–48 h to ensure confluence. HUVEC were identified by their typical cobblestone morphology and immunofluorescence staining by monoclonal antibodies against von Willebrand Factor (Immunotech, Milano, Italy). Cells up to the fourth passage were used for all experiments.

The investigation conformed with the principles outlined in the declaration of Helsinki for the use of human tissue.

2.2.2. PBMC

PBMC were isolated either from fresh buffy coats obtained from the local blood bank or from the peripheral blood of normal volunteers as described (Cerri et al., 2006). Briefly, after obtaining the necessary consent from the donor, a fresh buffy coat was mixed gently with an equal volume of 2.5% Dextran T500, and left for 40 min for erythrocyte sedimentation. Ten mL of leukocyte-rich supernatant was recovered and layered over 5 mL of Ficoll-Histopaque and centrifuged for 30 min at 350 ×*g* at 4 °C. The PBMC-rich ring was recovered and washed twice in phosphate buffered saline. PBMC were then resuspended in RPMI supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin, 2 mM L-glutamine and allowed to adhere for 30 min at 37 °C on 24-well plates (2×10^6 cells/well). Then the cells were washed two times with pre-warmed phosphate buffered saline and resuspended in RPMI, 100 U/mL penicillin, 100 μg/mL streptomycin, 2 mM L-glutamine, 5%FBS and incubated overnight at 37 °C.

All procedures related to the use of human blood and tissues were approved by the ethical committee of the Azienda Ospedaliero-Universitaria Pisana, Pisa, Italy (protocol number 16715).

2.3. MP generation and purification

PBMC and HUVEC were washed twice with pre-warmed phosphate buffered saline. For MP release analysis, SRM1648a was resuspended in cell media and added; after the time of incubation indicated in the relevant figures at 37 °C the supernatants were recovered, cleared by centrifugation at 14,000 ×*g* for 5 min at room temperature to remove dead cells and big cell fragments that might have detached during the stimulation and immediately used for further experiments. In selected experiments, MP were further purified by ultracentrifugation (100,000 ×*g* for 2 h, 4 °C); the pellet was resuspended in 250 μL of normal saline and used in an one-stage clotting assay to measure TF-dependent coagulation. For Nanosight analysis, an aliquot of cell medium was centrifuged at 4 °C at 1000 ×*g* for 15 min, at 2000 ×*g* for 15 min and then at 3000 ×*g* for 15 min. Supernatants were then submitted to ultracentrifugation at 110,000 ×*g* for 2 h at 4 °C.

2.4. Measurement of MP-associated phosphatidylserine (PS)

PS-positive MP in each sample were detected using the Zymuphen MPactivity kit (Hyphen BioMed, Neuville-sur-Oise, France) according to the manufacturer's instructions and expressed as PS concentration (nM PS).

2.5. Nanosight detection of MP

The number and dimension of MP were assessed by nanoparticle tracking analysis (NTA). Using a Nanosight LM10-HS system (NanoSight

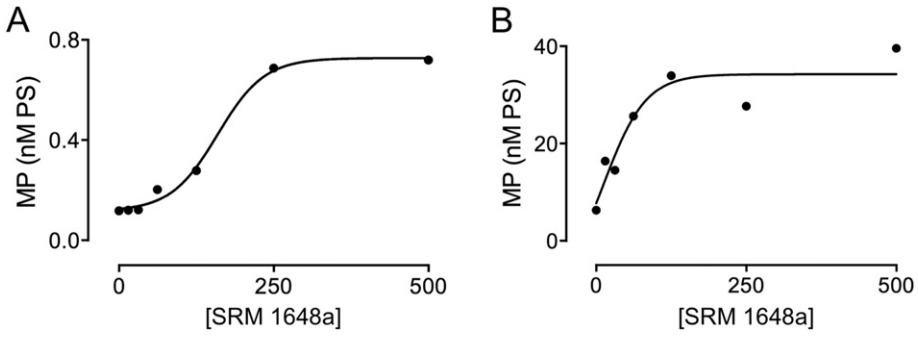

Fig. 1. Dose response curve of MP generation by PBMC (A) and HUVEC (B) upon incubation with SRM1648a (1 h). Data from one experiment representative of three for each cell type.