Mutation Research 713 (2011) 18–31

Contents lists available at ScienceDirect

Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis

journal homepage: www.elsevier.com/locate/molmut
Community address: www.elsevier.com/locate/mutres

Airborne urban particles (Milan winter-PM2.5) cause mitotic arrest and cell death: Effects on DNA, mitochondria, AhR binding and spindle organization

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ARTICLE INFO

Article history:
Received 22 October 2010
Received in revised form 13 May 2011
Accepted 20 May 2011
Available online 30 May 2011

Keywords:
Particulate matter
DNA-damage
Mitotic arrest
Cell death
Apoptosis

ABSTRACT

Airborne particulate matter (PM) is considered to be an important contributor to lung diseases. In the present study we report that Milan winter-PM2.5 inhibited proliferation in human bronchial epithelial cells (BEAS-2B) by inducing mitotic arrest. The cell cycle arrest was followed by an increase in mitotic-apoptotic cells, mitotic slippage and finally an increase in "classical" apoptotic cells. Exposure to winter-PM10 induced only a slight effect which may be due to the presence of PM2.5 in this fraction while pure combustion particles failed to disturb mitosis. Fewer cells expressing the mitosis marker phospho-histone H3 compared to cells with condensed chromosomes, suggest that PM2.5 induced premature mitosis. PM2.5 was internalized into the cells and often localized in laminar organelles, although particles without apparent plasma membrane covering were also seen. In PM-containing cells mitochondria and lysosomes were often damaged, and in mitotic cells fragmented chromosomes often appeared. PM2.5 induced DNA strands breaks and triggered a DNA-damage response characterized by increased phosphorylation of ATM, Chk2 and H2AX; as well as induced a marked increase in expression of the aryl hydrocarbon receptor (AhR)-regulated genes, CYP1A1, CYP1B1 and AhRR. Furthermore, some disturbance of the organization of microtubules was indicated. It is hypothesized that the induced mitotic arrest and following cell death was due to a premature chromosome condensation caused by a combination of DNA, mitochondrial and spindle damage.

1. Introduction

A number of epidemiological studies have suggested that airborne particulate matter (PM), especially the fine particles (PM2.5: aerodynamic diameter < 2.5 μm), increases morbidity and mortality from cardiopulmonary diseases [1–3]. PM may cause release of inflammatory mediators [4–6] and has been found to be genotoxic and cause cell death in various lung cells [7–9]. Such events are considered to be important contributors to lung diseases.

The understanding of PM-induced toxicity is complicated by the fact that PM is a very heterogeneous and often poorly described pollutant that varies with seasons and between regions. Particle size appears to be one of the critical parameters. However, the different size fractions also contain different types of particles. Although there is a certain overlap, the coarse fraction (PM10-2.5) is dominated by mechanically generated particles whereas PM2.5 is dominated by combustion particles from various sources. In addition, the PM composition may vary considerably depending on sampling season, region and sources [10–13]. As a consequence, the type of toxic responses induced by different PM samples may vary greatly. Notably, experimental studies have suggested that coarse PM induces mainly inflammatory responses due to crustal and biogenic elements [4,14–16] whereas fine PM rather increases DNA damage and cell death [17,18]. In a recent study we found that Milan winter-PM, and in particular the PM2.5 fraction, caused mitotic arrest in the human lung epithelial cell line BEAS-2B [18]. The cells arrested in mitosis seemed to die by a type of "mitotic-apoptosis" as well as a more "classical" apoptotic cell death. Since the induced mitotic arrest was correlated to a high level of polycyclic aromatic hydrocarbons (PAHs), we wanted to explore if DNA damage could be a part of the initiating signal.

During cancer development DNA damage and cell cycle perturbations are parallel ongoing events. The relative proportion of cell death versus cell survival signals is an important determinant influencing DNA repair and replication bypass fidelity, thus influencing the final mutagenic outcome [19,20]. Under "normal"

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doi:10.1016/j.mrfmmm.2011.05.011

conditions replication of damaged DNA is avoided by G1-arrest, whereas G2-arrest prevents entry into mitosis. Chromatin changes such as double strand breaks and single strand breaks activates the kinases *ataxia telangiectasia mutated* (ATM) and *ATM and Rad3 related* (ATR), respectively, and cell cycle is ultimately arrested by checkpoint kinases (Chk). ATM activates Chk2, while ATR activates Chk1. ATM and ATR may also activate p53 directly or indirectly through Chk1/2, ultimately triggering apoptosis [21,22]. Apoptosis is usually executed by caspases and morphologically characterized by cell shrinkage, nuclear and cytoplasmic condensation and fragmentation [23,24] and is often referred to as programmed cell death (PCD) based on need for protein synthesis. If the G2/M check-points fail to arrest cells with DNA damage in G2, the spindle checkpoint may arrest the cells in mitosis. Mitotic arrest may result in cell death during mitosis or following mitotic slippage [25]. Cell death during mitosis or after mitotic slippage is often termed mitotic catastrophe, an atypical mode of cell death which is due to premature or inappropriate entry into mitosis. There is however no clear biochemical definition of this form of cell death, and mitotic catastrophe may lead to either apoptotic or necrotic cell death [21–23,26].

PM, and in particular the fine PM fraction, contains a variety of chemicals which may damage DNA directly or interfere with DNA repair/synthesis such as PAHs and various metal components. Some of these compounds may also interfere with cell cycle checkpoints and apoptosis processes enabling cells with DNA damage to divide. Both metal and PAHs exposure may lead to formation of reactive oxygen and nitrogen species (ROS and RNS) which are both DNA reactive by themselves and also generate a number of other reactive molecules [27–30]. PAHs are also known to be activated into various reactive electrophilic metabolites covalently binding to macromolecules including DNA [9]. Important and well characterized effects of the PAHs are mediated through the binding to the cytosolic aryl hydrocarbon receptor (AhR) and subsequently increasing the expression of the CYP1A1 and -1B1 genes. Recent studies has suggested that some PAHs, through AhR activation, also may change the lipid synthesis with implication for the plasma membranes composition of lipids, thereby changing inter- and intra-cellular signaling pathways involved in cell death [31].

In the present study, we have further examined the effects of winter-PM2.5 and -PM10 from Milan on mitotic arrest and cell death in the human lung epithelial cell line BEAS-2B and explored possible mechanism involved. We found that while Milan winter-PM2.5 resulted in cell death either during mitotisis ("mitotic-apoptotic" cells) or directly after cell division ("classic apoptotic" cells), no such effects were seen after exposure to diesel exhausted PM and wood smoke PM. Milan winter-PM2.5 was found to elicit DNA and mitochondrial damage and to trigger DNA-damage response. Very marked changes in the expression of AhR related genes were seen and the spindle organization was also somewhat disturbed.

2. Material and methods

2.1. Chemicals

Saponin, Triton X-100, propidium iodide (PI), Hoechst 33342 and 33258 were obtained from Sigma–Aldrich, St. Louis, MO, USA. LHC-9 medium was purchased by Invitrogen (Burlington, ON), foetal calf serum (FCS) and gentamycin were from Gibco BRL (Paisley, Scotland, UK). DAPI-mounting medium and Alexa 488 goat anti-mouse IgG were from Molecular Probes (Eugene, OR). Caspase-3 primary polyclonal antibody and CYP1A1 Ab were purchased form Santa Cruz Biotechnology, Phospho-Histone H3 Alexa Fluor Red conjugate, p53 polyclonal primary antibody, pATM, pATR, Chk1, Chk2, and γ-H2AX primary antibody were from Cell Signaling (Beverly MA), 8-oxo-dG antibody was purchased from Trevigen (Gaithersburg, MD). All other chemicals were purchased from commercial sources at the highest purity available.

2.2. Preparation of particle samples

Samples of PM10 and PM2.5 were collected at Torre Sarca, a site in the Milan area considered representative of background urban atmospheric pollution concentrations in 2008 and 2009 during winter season. Low volume gravimetric samplers were used (EU system 38,33 l min^{-1}, FAI Instruments, Rome; Italy) and the atmospheric sampling point was at about 3 m from the ground. PM samples were collected on Teflon filters (Ø47 mm, 2 μm, Pall Gelman, USA) or quartz filters for chemical characterization. Before and after sampling, filters were 48 h-equilibrated (35% RH, T ambient) and weighted with a microbalance with precision of 1 μg (model M5P-000V001 Sartorius, Germany) to determine atmospheric particles concentration (μg/m^3). All sampled filters were then preserved in the dark at −4 °C (avoiding photodegradation and evaporation losses) for further chemical and biological analyses.

For the biological testing PM10 and PM2.5 were detached from Teflon filters by an ultrasound bath (SONICA, Soltec) in sterile water, dried and resuspended in sterile water at a final concentration of 4 μg/μl and stored at −20 °C.

Diesel exhaust PM (DEP) Reference DEP, SRM 1650a (National Institute of Standards and Technology (NIST), Gaithersburg, MD, USA) was suspended in sterile water at the final concentration of 2 μg/μl and then used to treat the cells.

Wood smoke PM (WSP) was sampled and characterized [32] and prepared as previously reported [33]. Briefly, WSP was washed with methanol to inactivate biogenic components. Methanol was then evaporated under a N_2 flux and the resulting WSP pellet suspended in sterile water at a final concentration of 2 μg/μl.

2.3. Cell culture

BEAS-2B cells, a SV40 hybrid (Ad12SV40) transformed human bronchial epithelial cell line, were purchased from American Tissue Type Culture Collection (ATCC, Rockville, MD, USA). The p53 of this cell line is mutated in codon 47 but this does not change its functional properties [34,35]. The cell line was maintained in LHC-9 medium at 37 °C with 5% of CO_2, split every 3 day and the medium was changed the day after. For experiments, cells were seeded at a concentration of 80,000 cells/well in 6-well plates and treated after two days with different concentrations (1, 10 and 25 μg/cm^2) of winter PM10 and PM2.5.

2.4. Viability and proliferation

Cytotoxicity was determined by Hoechst 33342/propidium (PI) staining as previously described [19]. Briefly, the cell growth medium was harvested to collect detached, floating cells, whereas attached cells were collected by trypsination. Trypsinised cells and the harvested medium were pooled, centrifuged, re-suspended in medium and stained with Hoechst/PI. Smears were prepared and cells were scored as viable normal cells (Hoechst stained and PI negative, without special nuclear characteristics and an intact plasma membrane), necrotic cells (non-apoptotic and PI positive), apoptotic cells (bright Hoechst/or PI positive stained with condensed or fragmented nuclei), mitotic cells (Hoechst positive with chromosome condensation) or mitotic-apoptotic cells (Hoechst positive with condensed chromosome resembling apoptotic features or clearly fragmenting chromosomes). Viable cells, without special nuclear characteristic, as well as normal mitotic cells were consid-