Toxicology in Vitro 25 (2011) 1132–1142

Contents lists available at ScienceDirect

Toxicology in Vitro

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

Application of cellular biosensors for analysis of bioactivity associated with airborne particulate matter

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ARTICLE INFO

Article history:
Received 4 December 2010
Accepted 25 March 2011
Available online 30 March 2011

Keywords:
Fluorescent Cell Chip
Oxibios
Airborne particulate matter
IL-2
IL-4
IFN-γ
IL-10
NFκB

ABSTRACT

Exposure to airborne particulate matter (PM) is a known risk factor for adverse health effects observed in many environmental and occupational settings. The pathological mechanisms involved in PM-mediated toxicity depend on the size and contents of particles that vary depending on the source of emission. Chemical compositions of PM show multiple components with different bioavailabilities that are capable of acting on multiple molecular and cellular targets, making it difficult to predict PM-associated toxicity based solely on chemical analysis. The aim of the study was to develop robust, sensitive and economical assays for environmental pollutants based on genetically modified mammalian cells. We tested the suitability of two biosensor assays, Fluorescent Cell Chip and Oxibios, developed in part in our laboratories, for assessment of the potential toxicity of airborne PM. Reference PM and PM obtained by sampling of diesel exhaust and indoor air in aluminum and copper facilities in Poland were tested with the two bioassays using unified experimental protocols. Resultant data showed complex patterns of stimulatory and inhibitory activities that were consistent with the origin of PM and might be correlated with their chemical composition. The analysis was informative with regard to type and extent of possible toxicity associated with specific PM and allowed for detection of significant differences between PM from different industrial sites and particular locations within the same industrial sites as well as overall ranking of toxicity risk based on chemical analysis.

1. Introduction

An important part of the routine assessment of the level of air pollution is determination of the amount of airborne particulate matter (PM) (Hauck, 1998). Epidemiological observations strongly support the causal relationship between the exposure to airborne PM and increased mortality and morbidity in exposed populations, mostly due to aggravation of respiratory and cardiovascular diseases (Katsouyanni et al., 2009; Polichetti et al., 2009; Romeo et al., 2006). While the overall amount of PM is an accepted marker of air quality, the risk of particular adverse health effects resulting from exposure to PM is strictly related to the source of emission which determines composition and size of particles (Aust et al., 2002; Chen and Lippmann, 2009; Schwarze et al., 2007). Typically, the chemical speciation of PM reveals organic and elemental carbon, polycyclic aromatic hydrocarbons, metals, transition metals, and other elements (Ntziachristos et al., 2007). It is difficult to link particular components of this complex mixture of organic and inorganic compounds with a specific biological activity of PM. There are examples in which the presence of certain components in PM might be correlated with observed biological activity. For example, the ability of PM to generate reactive oxygen species in exposed cells might be correlated with the presence of transition metals such as iron (Aust et al., 2002), and mutagenicity of PM observed in a standard bacterial assay was correlated with the presence of polycyclic aromatic hydrocarbons (de Aragao Umbuzeiro et al., 2008). While some activities of PM such as the overall redox activity could be directly assessed in a simple assay and attributed to the major components of PM (Ntziachristos et al., 2007), certain more subtle biological effects mediated by PM might result from the presence of a minor amount of a particular component and are difficult to determine in a single assay. Some of the minor components of PM such as lead and mercury (Vassilakos et al., 2007) are known to affect cytokine expression and other functions of immune cells *in vitro* and *in vivo* (Walczak-Drzewiecka et al., 2003; Shen et al., 2001), suggesting possible immunotoxicity that is difficult to determine in a single *in vitro* assay (Carfi et al., 2007). These examples suggest that prediction of various toxic effects mediated

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doi:10.1016/j.tiv.2011.03.019

by PM based solely on chemical analysis might be difficult. Employment of living mammalian cells for testing the bioactivities associated with PM *in vitro* may be useful not only for mechanistic studies but also for routine toxicity testing. In regard to this, there are multiple efforts to develop robust, sensitive and economical assays for environmental pollutants based on different types of biosensors including genetically modified mammalian cells (Pancrazio et al., 1999). We have decided to test the suitability of two cell-based biosensor assays, Fluorescent Cell Chip (Ulleras et al., 2005) and Oxibios, developed in part in our laboratories, for assessment of the potential toxicity of airborne PM. For this purpose, reference PM and PM obtained by sampling of diesel exhaust in The Netherlands and indoor air in two industrial facilities in Poland were tested in parallel with the two bioassays using unified experimental protocols. In both assays we observed reproducible responses that significantly varied in direction and intensity depending on PM origin and correlated with elemental contents of PM samples.

2. Materials and methods

2.1. Chemicals and reagents

Media for cell culture were purchased from Invitrogen, fetal calf serum FCS was obtained from Biowest, geneticin (G418) was purchased from Biochrom AG and hygromycin was purchased from Invivogen. All other chemicals were of analytical grade and obtained from Sigma Chemical Co. unless otherwise stated.

2.2. Collection, analysis and preparation of PM

Standard Reference Material 2584 (trace elements in indoor dust, nominal 1% lead), Standard Reference Material 2585 (organic contaminants in house dust), Standard Reference Material 1648 (urban particulate matter), Standard Reference Material 1649b (urban dust) and Standard Reference Material 1650b (diesel particulate matter) were purchased from the National Institute of Standards and Technology, Gaithersburg, MD, USA. Airborne PM in the aluminum manufacturing plant was collected in two sites, the casting and electrorefining departments, and sieved through a 36-μm mesh strainer. Airborne PM in the copper manufacturing plant was collected in two locations within anode furnace departments (sites A and D) and sieved mechanically through a 25-μm mesh strainer. Diesel particulate matter (DPM) was harvested in the National Institute for Public Health and the Environment (RIVM) in Bilthoven, The Netherlands, using an idling diesel engine (Cassee et al., 2002) and was a kind gift from Dr. Flemming Cassee. The analysis of elements of PM from aluminum and copper plants was performed in the ICP-MS laboratory of the Chemistry Faculty of Warsaw University. In brief, PM samples were dissolved in 63% nitric acid (with hydrofluoric acid addition) and 37% hydrochloric acid followed by mineralization in a closed microwave system. Decomposed samples were diluted with distilled water and analyzed using ICP-MS (inductively coupled plasma mass spectrometry; Elan 6100, Sciex Perkin Elmer) and F-AAS (flame atomic absorption spectrometry; AAnalyst 300, Perkin Elmer). Particle size distribution measurements for PMs were carried out using a laser diffraction instrument Mastersizer (Malvern Instruments Ltd., UK) in National Medicines Institute, Poland. A suspension of 10 mg/ml of PM in distilled water was analyzed before and after 1 h of sonication. Particle size was presented as mean particle diameter $d(0.5)$ (parameter for the particle-size distribution indicating the particle size below which 50% of the volume is present). Prior to experiments with *in vitro* cultured cells, samples of PM were weighed, sterilized in a steam autoclave, suspended in a sterile culture medium and sonicated for 1 h.

2.3. FCC assay

Five EL-4 (mouse lymphoma cell line)-derived reporter cell lines stably transfected with a transgene consisting of promoter regions from mouse β-actin (E/008/009), IL-2 (interleukin-2; E/253/006), IL-4 (interleukin-4; E/452/002), IFN-γ (interferon-γ; E/552/003) and IL-10 (interleukin-10; E/752/005) and ORF (open reading frame) for EGFP (enhanced green fluorescent protein) were described earlier (Ulleras et al., 2005). Reporter cells were cultured at a density of 5×10^5–1×10^6 cells/ml in RPMI 1640 medium supplemented with 2 mM L-glutamine, 20% FCS and 0.5 μg/ml G418 in a humidified atmosphere with 5% CO_2 at 37 °C. The principles of the Fluorescent Cell Chip (FCC) assay were described earlier (Wagner et al., 2006). In brief, cells were seeded at a density of 0.5×10^5 per well in a 96-well plate and incubated with increasing concentrations of tested substance in a total volume of 100 μl at 37 °C in a humidified atmosphere of 5% CO_2 for 18 h. Each plate was arranged to accommodate five reporter cell lines, either activated with PMA (phorbol 12-myristate 13-acetate; 50 ng/ml) and ionomycin (1 μM) or not and exposed to the tested PM suspension at concentrations of 0, 5, 10, 25, 50, 100, 250 and 500 μg/ml. For each cell line there were two controls, one consisting of cells in medium only and another consisting of cells activated with PMA/ionomycin. Following the 18-h incubation, cells were analyzed by flow cytometry to determine the level of EGFP-mediated fluorescence and cell viability.

2.4. Flow cytometry

Before acquisition, the volumes of samples were adjusted to 250 μl with PBS. Cells were analyzed in the Cytomics FC 500 MPL Beckman-Coulter flow cytometer equipped with a multi-plate loader and MXP Acquisition Software v.2.1 (Beckman Coulter Inc.) The level of EGFP-mediated fluorescence of the reporter cells was measured after gating on live cells. In the routine acquisition protocol for determination of EGFP-mediated fluorescence, live cells were selected based on live/dead cell gates on an FS/SS plot. These gates were adjusted and established experimentally by comparing them with the ones set up upon testing a range of immunotoxic substances on EL-4/β-actin reporter cells and staining with dedicated fluorescent dyes: PI (propidium iodide) and 7-AAD (7-aminoactinomycin D). FS/SS gates vs. PI or 7-AAD gates showed good correlation with differences not exceeding ±5% of the analyzed cell population.

2.5. Development of the reporter cell lines for Oxibios assay

For construction of the NFκB-responsive reporter plasmid, an artificial promoter sequence was prepared as an *in vitro*-synthesized DNA oligonucleotide. This promoter sequence contained a 6× concatenated consensus NFκB binding site (5′-GGGAATTTCC-3′) and a minimal core promoter derived from the adenovirus E1b gene. The artificial promoter was cloned into the firefly luciferase-encoding pGL4.14 vector (Promega) and its specific inducibility by NFκB-activating stimuli was verified by transient transfection into several human cancer cell lines. Selection of cell lines for further stable transfection of the construct was based on strength and specificity of induction of transcriptional activity by classical NFκB-activating stimuli (TNFalpha, interleukin-1 and oxidative stress) with corresponding lack of induction by non-cognate signaling pathway activation. Due to presence of expressed receptors and intracellular components of the NFκB pathway, two cancer cell lines of different tissues of origin (ovarian epithelial – SK-OV-3 and hepatocellular – Hep3B) were found to be optimal for specific detection of NFκB activation. The developed vector was used for stable transfection into these cell lines with hygromycin antibiotic selection and clonal