Toxicology 303 (2013) 83–93

Contents lists available at SciVerse ScienceDirect

Toxicology

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

Gene expression profiling to identify potentially relevant disease outcomes and support human health risk assessment for carbon black nanoparticle exposure

Julie A. Bourdon[a,b], Andrew Williams[a], Byron Kuo[a], Ivy Moffat[a,b], Paul A. White[a], Sabina Halappanavar[a], Ulla Vogel[c], Håkan Wallin[c], Carole L. Yauk[a,*]

[a] Environmental Health Science and Research Bureau, Health Canada, 50 Columbine Drive, Tunney's Pasture, Ottawa, Canada K1A 0K9
[b] Water, Air and Climate Change Bureau, Health Canada, 269 Laurier, Ottawa, Canada K1A 0K9
[c] National Research Centre for the Working Environment, Lersø Parkallé 105, DK-2100, Copenhagen, Denmark

ARTICLE INFO

Article history:
Received 8 August 2012
Received in revised form 11 October 2012
Accepted 16 October 2012
Available online 9 November 2012

Keywords:
Toxicogenomics
Human health risk assessment
Nanoparticles
Systems biology
Mode of action
Hazard identification

ABSTRACT

New approaches are urgently needed to evaluate potential hazards posed by exposure to nanomaterials. Gene expression profiling provides information on potential modes of action and human relevance, and tools have recently become available for pathway-based quantitative risk assessment. The objective of this study was to use toxicogenomics in the context of human health risk assessment. We explore the utility of toxicogenomics in risk assessment, using published gene expression data from C57BL/6 mice exposed to 18, 54 and 162 μg Printex 90 carbon black nanoparticles (CBNP). Analysis of CBNP-perturbed pathways, networks and transcription factors revealed concomitant changes in predicted phenotypes (e.g., pulmonary inflammation and genotoxicity), that correlated with dose and time. Benchmark doses (BMDs) for apical endpoints were comparable to minimum BMDs for relevant pathway-specific expression changes. Comparison to inflammatory lung disease models (i.e., allergic airway inflammation, bacterial infection and tissue injury and fibrosis) and human disease profiles revealed that induced gene expression changes in Printex 90 exposed mice were similar to those typical for pulmonary injury and fibrosis. Very similar fibrotic pathways were perturbed in CBNP-exposed mice and human fibrosis disease models. Our synthesis demonstrates how toxicogenomic profiles may be used in human health risk assessment of nanoparticles and constitutes an important step forward in the ultimate recognition of toxicogenomic endpoints in human health risk. As our knowledge of molecular pathways, dose–response characteristics and relevance to human disease continues to grow, we anticipate that toxicogenomics will become increasingly useful in assessing chemical toxicities and in human health risk assessment.

1. Introduction

Chronic inhalation of fine and ultrafine particulate matter has been associated with adverse pulmonary effects including fibrosis and cancer, as well as exacerbation of existing conditions such as asthma, bronchitis and chronic obstructive pulmonary disorder (Bonner, 2007; Knaapen et al., 2004), in addition to cardiovascular disease (Dockery et al., 1993; Pope et al., 2004). Human exposure to manufactured nanomaterials (NMs), which have at least one size dimension that is less than 100 nm, may constitute an increased risk of adverse effects especially following inhalation exposure, and their potential to induce toxic effects is poorly understood (Handy and Shaw, 2007). Moreover, the human health risks associated with inhalation exposure have not been adequately investigated. Methods that can be effective in screening for NM toxicities are paramount, due to the countless variations in physical and chemical properties of NMs in terms of size, shape, agglomeration and surface coatings.

Traditional assays used in human health risk assessment (HHRA) generally involve chronic and subchronic rodent exposures with concomitant analyses of tumour induction (e.g., two-year rodent cancer bioassay), in addition to various non-cancer endpoints, the most sensitive of which is used for regulatory decision-making (Meek et al., 1994). These approaches form the foundation of the chemical regulatory system and have been invaluable for HHRA. However, some of these assays, such as those based on chronic animal exposures at the maximum tolerated dose, are time and resource intensive, thus limiting broad application (Suter et al., 2004). Recent discussions have identified gene expression profiling as a potentially rapid and cost-effective approach for identifying and assessing prospective hazard, characterizing chemical

Abbreviations: BMD, benchmark dose; BMDL, lower confidence limit benchmark dose; CBNP, carbon black nanoparticle; FDR, false-discovery rate; HHRA, human health risk assessment; NM, nanomaterial.

* Corresponding author. Tel.: +1 613 941 7376; fax: +1 613 941 8530.
E-mail address: Carole.Yauk@hc-sc.gc.ca (C.L. Yauk).

0300-483X/$ – see front matter.
http://dx.doi.org/10.1016/j.tox.2012.10.014

(or particle) mode of action, and assessing human relevance in support of HHRA (National Academy of Sciences, 2007). In order for gene expression data to become accepted for routine use in HHRA, it is necessary to demonstrate that mRNA/protein expression profiles can effectively predict the modes of action and biological outcomes of exposure at relevant doses, and to confirm that these data can be used to strengthen the foundation for HHRA and regulatory decisions. In this regard, it has been hypothesized that gene expression profiling will be extremely useful in identifying effects at low doses, and moreover, useful for distinguishing between doses that elicit an adaptive response vs. those that yield adverse effects (Boverhof and Zacharewski, 2006). To date, the application of gene expression profiling in regulatory toxicology has largely focused on qualitative identification of chemical modes of action and transcription biomarkers that can predict specific toxicities. However, the utility of gene expression profiling in quantitative determination of threshold values (e.g., benchmark doses) has not yet been rigorously explored (Thomas et al., 2012).

In the present study we investigate the utility of gene expression profiles derived from mice exposed to Printex 90 carbon black nanoparticles (CBNPs) by intratracheal installation to identify potential hazards, modes of action, and doses above which adverse effects may be expected for specific toxicological outcomes. In addition, we quantitatively compare benchmark doses for pathways to those of apical endpoints derived from the same experimental animals. We employ Printex 90 as a model NM due to the rich database of traditional toxicity information on which our findings can be anchored. Briefly, Printex 90 consists almost entirely of carbon, with very low levels of impurities in terms of polycyclic aromatic hydrocarbons and endotoxins (Bourdon et al., 2012b; Jacobsen et al., 2008; Saber et al., 2011) They generate reactive oxygen species (Jacobsen et al., 2008), induce DNA strand breaks in vitro and in vivo (Jacobsen et al., 2009; Saber et al., 2005) and mutations in vitro (Jacobsen et al., 2007) that are associated with oxidative stress (Jacobsen et al., 2011). The data in this study are from previously published experiments investigating Printex 90 CBNP exposure in C57BL/6 mice at various doses (i.e., vehicle, 18, 54 and 162 μg) collected at several time-points (1, 3 and 28 days) following a single acute instillation (Bourdon et al., 2012a). We previously characterized widespread changes in gene expression involving acute phase response and inflammation, supported by concomitant influxes of pulmonary bronchoalveolar lavage cells (BAL) and increases in tissue-specific DNA strand breaks (Bourdon et al., 2012a,b). In addition to the examination of BMDs and BMDLs, we compare CBNP-modified gene expression profiles to various models of lung disease in mice and humans reported in the literature, in order to explore the utility of our data in predicting the potential risk of adverse health outcomes and the human relevance of expression changes. The work demonstrates one approach by which gene expression profiling may be integrated into HHRA to support or predict apical toxicological endpoints, dose–response, and relevance to human diseases.

2. Materials and methods

2.1. Summary of experimental model and published results

Details of the mouse exposures, particle characterization and pulmonary phenotype were previously published in Bourdon et al. (2012a,b). Briefly, female C57BL/6 mice were exposed to a single installation of vehicle or Printex 90 (18, 54 or 162 μg) and euthanized 1, 3 and 28 days post-exposure ($n = 6$/group). The intratracheal instillation route of exposure allows for deposition of known doses directly in the lungs of the mice, and controls for potential dermal- and ingestion-related CBNP exposure that can occur during whole body inhalation exposures. The doses were selected to represent 1, 3 and 9 working days of exposure at the occupational inhalation exposure limit of 3.5 mg/m^3 of CB (as established by the US Occupational Safety and Health Administration (OSHA) and the US National Institute for Occupational Safety and Health (NIOSH))) for a mouse (assuming 1.8 L/h inhalation rate and 33.8% particle deposition in mouse, for an 8 h working day) (Dybing et al., 1997; Jacobsen et al., 2009). Very limited filtration of CBNPs from the nose is expected during human exposure. Printex 90 CBNPs were characterized and displayed the following properties: 14 nm primary particle size, 295–338 m^2/g Brunauer Emmett and Teller (BET) surface area, 74.2 μg/g PAHs, 142 EU/g endotoxin, polydispersity index of 1, −10.7 mV zeta potential, 2.6 μm peak hydrodynamic number and 3.1 μm peak volume-size-distribution (Bourdon et al., 2012b).

Analysis of pulmonary inflammatory cellular influx in bronchoalveolar lavage (BAL) revealed neutrophilic inflammation that was sustained to day 28 at all doses. Tissue-specific genotoxicity, as observed by DNA strand breaks, persisted up to day 28 at the two highest doses and FPG-sensitive sites at all doses on day 1 and the highest dose on day 3 (Bourdon et al., 2012b). Whole mouse genome DNA microarray revealed 487 and 81 differentially expressed genes (FDR adjusted p-value ≤ 0.1 and fold changes ≥ 1.5) overall in lung and liver, respectively (Bourdon et al., 2012a). The complete microarray dataset is available through the Gene Expression Omnibus at NCBI (http://www.ncbi.nlm.nih.gov/geo/, Superseries GSE35284, SubSeries GSE35193). This dataset was previously used to examine molecular interactions between lung and liver upon CBNP exposure (Bourdon et al., 2012a).

2.2. Rank-based pathway analysis

To determine the most affected processes of CBNP exposure, pathway analysis of gene expression data was conducted using a rank based test in R (R Development Core Team, 2011) as described in Alvo et al. (2010). The relative expression for the genes in a pathway was first aligned by subtracting the median expression value for the combined treatment and control groups. These values were then ranked within each subject and the vector of average ranks was calculated for each treatment group. The distance between the two treatments was calculated and a permutation analysis was used to obtain a p-value for each pathway. Pathways with $p < 0.05$ were considered significant.

2.3. Benchmark dose (BMD)/lower confidence limit benchmark dose (BMDL) calculation for apical endpoints and RT-PCR data

BMD_{10} (BMD representing an excess risk of 10% in exposed animals vs. controls) and BMDLs (95% confidence limit) were calculated for apical endpoint data (inflammation and genotoxicity) and for RT-PCR using EPA BMDS 2.2 (Davis et al., 2011). Only data that were statistically above control levels ($p < 0.05$) for at least two of the doses were included. Prior to running the analysis, the data were screened for homogeneity of variance, and then fit against five continuous dose–response models (i.e., hill, polynomial, linear, power and exponential). Goodness of fit >0.05 and scaled residuals within ±2.0 was applied as a cut off for selection of the appropriate model, and curves were also inspected visually. When more than one model was suitable, the one with the lowest Akaike's information criterion (AIC) was selected.

2.4. BMD/BMDL calculation for genomic data

In order to determine BMDs and BMDLs for gene expression data, BMDExpress was employed (Yang et al., 2007). Briefly, microarray probes with more than one representation on each array were averaged. Analyses were performed on genes that were identified as statistically significant by one-way ANOVA ($p < 0.05$) using the four following models: Hill, Power, Linear 1° and Polynomial 2°. The Power model had a power restriction of ≥1. Selection on Linear and Polynomial 2° was based on choosing a model which describes the data with the least complexity. A nested Chi-square test, with cut-off of 0.05, first selects among linear and polynomial models, followed by comparing AIC, which measures the relative goodness of fit. A Hill model was excluded if the "k" parameter of the model was less than 1/3 of the lowest positive dose (18 μg) (Black et al., 2012). Other settings included maximum iterations of 250, confidence level of 0.95, benchmark response (BMR) of 1.349 (number of standard deviation defining BMD) (Yang et al., 2007). For functional classifications and analyses, the resulting BMD datasets were mapped to KEGG pathways with promiscuous probes removed (probes that mapped to multiple annotated genes). BMDs that exceeded the highest exposure dose (162 μg) and that exceeded a goodness-of-fit p-value of 0.1 were removed from the analysis.

2.5. Prediction analysis for microarrays

To determine the correlation between gene expression profiles of mice exposed to CBNPs with those of mouse pulmonary disease models, a prediction analysis for microarrays (PAM) (Tibshirani et al., 2002) was conducted in R (R Development Core Team, 2011) using the PAMR library (Hastie et al., 2011). Data for this analysis encompassed 13 mouse lung disease models, and were obtained from the National Centre for Biotechnology Information Gene Expression Omnibus (accession #GSE4231 and #GSE11037). The samples were labelled as belonging to one of three models of lung inflammation: bacterial infection, lung injury and fibrosis, or Th2 response (allergic airway inflammation). Probes with common GENBANK accessions were collapsed to a single measurement for each sample using the mean. Using the common accession numbers, a prediction model using shrunken centroids was estimated. Cross-validation of the nearest shrunken centroid classifier was conducted to identify an appropriate threshold. PAMR implements 10-fold cross-validation. This involves dividing the samples into ten approximately