Experimental and Toxicologic Pathology 65 (2013) 1063–1071

Contents lists available at ScienceDirect

Experimental and Toxicologic Pathology

journal homepage: www.elsevier.de/etp

Gene expression changes in rat brain after short and long exposures to particulate matter in Los Angeles basin air: Comparison with human brain tumors

Julia Y. Ljubimova[a,*], Michael T. Kleinman[b], Natalya M. Karabalin[a], Satoshi Inoue[a], Bindu Konda[a], Pallavi Gangalum[a], Janet L. Markman[a], Alexander V. Ljubimov[c], Keith L. Black[a]

[a] Department of Neurosurgery, Cedars-Sinai Medical Center, Los Angeles, CA 90048, United States
[b] Department of Community and Environmental Medicine Air Pollution Health Effects Laboratory, University of California, Irvine, CA 92697, United States
[c] Department of Surgery and Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA 90048, United States

ARTICLE INFO

Article history:
Received 9 January 2013
Accepted 19 April 2013

Keywords:
Air pollution particles
Chronic exposure
Gene microarray
Brain tumor
Arc/Arg3.1
Rac1

ABSTRACT

Air pollution negatively impacts pulmonary, cardiovascular, and central nervous systems. Although its influence on brain cancer is unclear, toxic pollutants can cause blood–brain barrier disruption, enabling them to reach the brain and cause alterations leading to tumor development.

By gene microarray analysis validated by quantitative RT-PCR and immunostaining we examined whether rat ($n = 104$) inhalation exposure to air pollution particulate matter (PM) resulted in brain molecular changes similar to those associated with human brain tumors. Global brain gene expression was analyzed after exposure to PM (coarse, 2.5–10 μm; fine, <2.5 μm; or ultrafine, <0.15 μm) and purified air for different times, short (0.5, 1, and 3 months) and chronic (10 months), for 5 h per day, four days per week. Expression of select gene products was also studied in human brain ($n = 7$) and in tumors ($n = 83$).

Arc/Arg3.1 and *Rac1* genes, and their protein products were selected for further examination. Arc was elevated upon two-week to three-month exposure to coarse PM and declined after 10-month exposure. Rac1 was significantly elevated upon 10-month coarse PM exposure. On human brain tumor sections, Arc was expressed in benign meningiomas and low-grade gliomas but was much lower in high-grade tumors. Conversely, Rac1 was elevated in high-grade vs. low-grade gliomas. Arc is thus associated with early brain changes and low-grade tumors, whereas Rac1 is associated with long-term PM exposure and highly aggressive tumors. In summary, exposure to air PM leads to distinct changes in rodent brain gene expression similar to those observed in human brain tumors.

1. Introduction

The American Cancer Society states that the incidence of brain tumors in the USA is growing, with an estimated 22,900 individuals diagnosed with malignant tumors of the brain or spinal cord in 2012. Brain cancer accounts for about 1.3% of all cancers and 2.2% of all cancer-related deaths for both adults and children. Moreover, brain and spinal cord tumors are the second most common cancers in children after leukemia, accounting for about 21% of childhood cancers. Around 3400 central nervous system tumors are diagnosed each year in children under the age of 20 (http://www.cancer.org/cancer/braincnstumorsinadults/detailed-guide/brain-and-spinal-cord-tumors-in-adults-key-statistics).

The most frequent types of human brain tumors are gliomas, meningiomas, and pituitary adenomas. The etiology of human brain tumor development is largely unknown. Epidemiologic evidence supports the importance of both genetic predisposition and environmental factors including air pollution in this process (Inskip et al., 1995). However, possible connections between the increase in brain tumor incidence and worsening of pollution in the industrial areas are unclear.

Exposure to air pollution has been associated with respiratory, cardiovascular, and stroke-related problems and death (Banauch et al., 2006; Calderón-Garcidueñas et al., 2003a; Villarreal-Calderon et al., 2012; Brunekreef and Holgate, 2002; Corea et al., 2012). Air pollution is a complex and dynamic mixture of gasses, particulate matter (PM), and organic compounds present in outdoor and indoor air. The size and chemical composition of PM depends on is source and also on atmospheric chemistry. Ultrafine particles (UFP; particle diameter ≤ 0.1 μm) are predominantly formed during combustion, by nucleation of vapor phase pollutants or by gas to particle conversions induced by chemical and photochemical reactions in the atmosphere. Fine particles (FP; particle diameter ≤ 2.5 μm) are

* Corresponding author at: Nanomedicine Research Center, Department of Neurosurgery, Cedars-Sinai Medical Center, 8631 West Third Street, Suite 800E, Los Angeles, CA 90048, United States. Tel.: +1 310 423 0834; fax: +1 310 423 0810.
E-mail addresses: ljubimovaj@cshs.org, Kymberly.Moore@cshs.org (J.Y. Ljubimova).

0940-2993/$ – see front matter © 2013 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.etp.2013.04.002

released by automotive and industrial emissions and are formed by condensation and coagulation processes in the air. Coarse particles (CP; 2.5 ≤ particle diameter ≤ 10 μm) are produced by mechanical processes (e.g., abrasion of brake linings and resuspension by wind).

The available evidence suggests a link between air pollution and brain tissue damage. Calderón-Garcidueñas's group identified pollution-related cognitive deficits, neuroinflammation, elevated levels of certain pro-inflammatory factors, neurodegeneration, and neurological injury including DNA damage in the brains of Mexico City dogs (Calderón-Garcidueñas et al., 2003b, 2007, 2008a). They also found the same alterations as well as Alzheimer's-like pathology, and disruption of the blood–brain barrier (BBB) in Mexico City children and young adults exposed to heavy urban pollution (Calderón-Garcidueñas et al., 2004, 2008a,b, 2012a,b). Tissue from the brains of pollution-exposed Mexico City residents showed signs of damage that could result from PM-induced formation of reactive oxygen species (ROS), effects of particulate-matter-associated lipopolysaccharides (PM-LPS), and/or effects of metallic components of inhaled PM (Calderón-Garcidueñas et al., 2012b; Block and Calderón-Garcidueñas, 2009).

Clinically healthy, cognitively and neurologically intact children and young adults with a lifetime exposure to elevated air pollutants including O_3, PM, and PM-LPS exhibit mRNA upregulation for cyclooxygenase-2 (COX-2), interleukin (IL)-1α, tumor necrosis factor (TNF)-α, and a key innate immunity receptor CD14 in the olfactory bulb, frontal cortex, substantia nigrae, and/or vagus nerves, as well as early disruption of the tight junctions in frontal blood vessels, which are the structural components of the BBB (Calderón-Garcidueñas et al., 2003a; Block and Calderón-Garcidueñas, 2009; Abbott, 2005; Campbell et al., 2009). Importantly, IL-1α has a prominent function in the self-propagation of neuroinflammation (Xiao et al., 2006; Blamire et al., 2000; Cunningham et al., 2005; Ferrari et al., 2006; Minghetti, 2005; Allan et al., 2005), BBB disruption, recruitment of inflammatory cells into the CNS (Ferrari et al., 2006), sustained upregulation of IL-8, VCAM-1, and ICAM-1 in astrocytes (Moynagh, 2005), and with neuronal, glial, and endothelial injury.

The routes by which PM components can enter the brain are not fully understood, but there is some evidence that UFP and toxic metals can traverse from the nasal epithelium to the brain via olfactory neurons. Brain effects could also be mediated by irritation of cranial nerves, such as the trigeminal and vagal. Other likely routes of brain exposure could be via the systemic circulation through an impaired BBB, and by macrophage-like cells loaded with PM from the lungs penetrating through lymphatic and systemic circulation systems to access the brain (Calderón-Garcidueñas et al., 2004, 2007, 2008b; Block and Calderón-Garcidueñas, 2009).

Although it is unclear how air pollution impacts brain tumor development (Terzano et al., 2010; Gerlofs-Nijland et al., 2010; van Berlo et al., 2010), some data indicate that this connection exists (Raaschou-Nielsen et al., 2011; Wu et al., 2012). It may be suggested that PM-induced inflammation could cause brain tissue damage triggering the activation of inflammatory cytokines that could alter signaling pathways associated with cell proliferation, cytoskeleton changes, and other molecular events associated with malignant transformation of brain cells. A large gene microarray study conducted here on brain tissues from rats exposed to airborne PM detected upregulation of genes associated with inflammation and that are also differentially expressed in human brain tumors. Several of these genes were selected as potential candidates for further validation in a series of PM exposure studies ranging from two weeks to ten months. We report on the PM exposure-related changes in the expression of specific genes and proteins in four rat experiments linked in terms of the source of ambient particles and exposure time, correlating them with the patterns of these potential markers in various human brain tumors.

2. Materials and methods

2.1. Animals and human tissues

All animals used in this study were Fischer 344 male rats and were between 3 and 7 weeks of age at the beginning of their exposure. They were obtained from National Cancer Institute (Bethesda, MD) or Harlan Laboratories (Livermore, CA). The animals were housed at the University of California, Irvine, where they were provided regular rodent lab chow and filtered water ad libitum and maintained on a 12/12-h light/dark cycle. The rats were housed in pairs on bedding material in ventilated cages supplied with filtered air. The study conformed to the "Guide for the Care and Use of Laboratory Animals" (NIH publication 85-23, revised 1996) and to the ethical standards of the 1964 Declaration of Helsinki, and was approved by the Institutional Animal Care and Use Committees at the University of California, Irvine (IACUC protocol # 2002-2242). Human brain tissues (normal brains from trauma patients, and brain tumors) were obtained from tumor collection of the Department of Neurosurgery at Cedars-Sinai Medical Center under an approved IRB protocol # 3646. Seven normal (non-tumor) specimens and 83 brain tumor specimens including 29 meningiomas, 24 low-grade gliomas and 30 high-grade gliomas (glioblastoma multiforme, GBM) were examined immunohistochemically.

2.2. Experimental procedures

2.2.1. Particle characterization

Samples were collected from ports immediately upstream of the exposure chambers during the exposures to determine physical and chemical characteristics of the UFP, FP, and CP exposure aerosols. Typically, particles from four days of exposure were collected per filter. For mass measurements, aerosol particles were collected on pre-weighed 25 mm Teflon filters (Zefluor 1-μm pore, Pall Corporation, Ann Arbor, MI). The Teflon filters were weighed after overnight equilibration (46% relative humidity, 20 °C) before and after each exposure session. For elemental and organic carbon analysis, particles were collected on 25 mm pre-baked quartz filters (Tissuequartz, Pall Corporation). At the end of each exposure day, quartz filter cassettes were removed, sealed, and stored under refrigeration. The cassettes were reinstalled the next day and sampling continued. After four days of exposure, the quartz filters were stored at −20 °C until they were analyzed. The speciation of elemental and organic carbons was determined using the thermal MnO_2 oxidation method by Atmoslytic Inc. (Calabasas, CA). Particle number concentrations of UFP and FP were measured using a TSI 3022 Condensation Particle Counter (TSI Incorporated, Shoreview, MN).

2.2.2. Exposure of animals to ambient particles

Rats were exposed to concentrated ambient particles for 5 h per day, 4 days per week for periods of 0.5, 1, 3 and 10 months in Riverside, CA. Riverside is characterized by high levels of particulate air pollution with a mixture of regional air pollution plus motor vehicle exhaust emissions from a nearby (~500 m) heavily trafficked major freeway interchange. The exposures were centered around the summer months, which are the times of greatest photochemical activity in Southern California. Exposures were performed from 8 AM to 1 PM; this 5-h exposure covered most of the morning and early afternoon peak exposure period. The 4 days per week pattern was selected because air pollution episodes in Riverside average 3–4 consecutive days. Detailed descriptions of the exposure methodology (Kleinman et al., 2005, 2007) and concentrator used in our study [Versatile Air Concentrator Enrichment System (VACES)] (Kim et al., 2001a,b) have been previously published. On each exposure day, rats were transported to Riverside from housing