Exposure to Nanoparticles Collected in the Los Angeles Basin in California Leads to the Upregulation of Brain-Associated Genes

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ABSTRACT

Direct evidence regarding brain tumors as a result of exposure to specific chemical agents and particulate air pollutants is limited.

We examined whether the different sizes of nanoparticles from Los Angeles basin in California, coarse (2.5-10 μm), fine (<2.5 μm), and ultra fine (<0.15 μm), were related to rat brain gene expression changes and compared the results obtained on animal tissues with data collected for human tumors.

The *Arc* gene was one of the gene microarray-selected genes studied in this investigation.

After two weeks of exposure, expression of the *Arc* gene increased in the coarse particle group. Animals exposed to particle-free air did not express *Arc* gene. The same result was obtained after one and three month' exposures.

On the basis of human brain tissue studies it has been previously speculated that *Arc* gene may play a role in benign and early malignant (grade II) tumor development.

Arc gene, a growth factor and cell growth activity-regulated gene, codes for a cytoskeleton-associated protein, whose expression has been detected here after exposure to air particles for the first time. *Arc* relationship to *c-fos* oncogene, the member of *Ras* oncogene family, is important because it is associated with tumor growth. *Arc* belongs to a group of "immediate early expressed genes" specific for early brain changes.

1 INTRODUCTION

The relationship between brain tumor occurrence and exposure to specific chemical agents and particulate air pollutants is unclear. This is in contrast to agents associated with tumors of the lung, colon, stomach, breast, uterus, and liver [1]. Furthermore, studies related to central nervous system (CNS) tumors tend to be largely dealing with occupational risks rather than exposure to particulate air pollution [2]. Occupation in the electrical and electronic fields, oil refining, rubber, airplane manufacture, machining, farming, pharmaceutical and chemical industries has been associated with increased CNS tumor risk [3].

We wanted to determine whether differences in the size of air particles collected in the Los Angeles basin in California, such as coarse (2.5-10 μm), fine (<2.5 μm), and ultra fine (<0.15 μm) particulate matter (PM), were related to the rat brain changes in gene expression level, and to compare the animal results with data for human tumors from our brain tumor tissue collection [4].

2 MATERIAL AND METHODS

2.1 Animal exposure

Groups of three to ten Brown Norway rats each were exposed through the breathing air to concentrated particulate matter (PM) nanoparticles. By size, the particles were either ultra fine (PM diameter <0.15 μm), fine (PM diameter <2.5 μm), or coarse (PM diameter 2.5-10 μm). Control rats were exposed to purified air. Exposures were for 5 hr per day, for 2 weeks, one month, and three months. The average exposure concentration was 600 $\mu g/m^3$. 24 hr after the final exposure, the rats were euthanized, their brains harvested, and mRNA purified to be used for gene chip analysis and Q-PCR.

2.2 Gene array analysis

Using Affymetrix GeneChip Rat Genome 230 2.0 microarray chips harboring probes for 28,000 genes was performed.

2.3 Quantitative RT-PCR (Q-PCR) analysis

The gene microarray data for *Arc* gene expression in individual rats were verified by Q-PCR. The Q-PCR reaction mixture contained the following: TaqMan Gene Expression Assay (Applied Biosystems) primers and probe for either *Arc* gene, or primers and probe for *GAPDH* as a housekeeping control gene, and TaqMan Universal PCR Master Mix in a total volume of 20 μl. Two TaqMan Gene Expression Assay sets for *Arc* gene were tested and gave very similar results. The thermal cycling conditions for Q-PCR were 50^0C for 2 min, 95^0C for 10 min, and 40 cycles of denaturing (95^0C for 15 sec) and annealing/ extension (60^0C for 1 min). PCR reactions were monitored in real time using the ABI PRISM 7700 Sequence Detector (Applied Biosystems). Controls without template were included for each primer set. Each experiment was carried out in duplicate. Q-PCR data were analyzed by two-tailed *t*-test (GraphPad Prism). $P<0.05$ was considered significant.

2.4 Immunohistochemistry

To examine the expression of Arc protein and its importance for brain molecular changes, sections of 15 human brain tumors were stained using three different anti-Arc antibodies

from Santa Cruz Biotechnology. Antibody E-19 (cat. no. sc-6382) gave the best results and was subsequently used in all the immunostaining experiments.

3 RESULTS

3.1 Gene array analysis

A two-fold cutoff ratio between data of control and experimental groups of animals was adopted as in most other studies by gene microarray analysis. This threshold yielded nine genes with significant changes in expression after particle exposure. Seven of them were upregulated and two were downregulated. The identity of two of the upregulated genes was already known. The other seven (two downregulated and five upregulated) belonged to the dsEST category (double stranded expressed sequence tags not currently assigned to known genes). One gene with increased expression after coarse particle exposure is *Arc* (GenBank accession number NM_019361).

3.2 Q-PCR Analysis

Two sets of *Arc* primers gave similar results, confirming *Arc* gene expression. After two weeks of exposure, expression of *Arc* was significantly increased in the coarse particle group. Ultra fine and fine particles induced lower but significant *Arc* gene expression. Clean air exposed animals did not express *Arc* gene. The same observations were obtained after one and three months of air particle exposure. However, *Arc* expression levels were the highest after two weeks. These data favor the hypothesis that *Arc* expression could be important for early "brain tissue injury" phase. Since *Arc* expression is regulated by the Ras pathway, the induction/stimulation of this pathway may be an immediate molecular response to tissue insult by particles.

3.3 Human brain tissue study

Arc was not expressed in normal human brain by immunohistochemistry. Moderate expression was detected in two out of four astrocytomas grade II. Weak expression was observed in two out of four astrocytomas grade IV, or glioblastomas multiforme. High Arc expression was detected in four meningiomas, the benign brain tumors. Based on this finding we can speculate that *Arc* gene, being part of Ras tumor signaling pathway, may play a role in benign and early malignant tumor development (grade II glial tumors); then its expression may decline during tumor progression towards grade IV glioma.

4 CONCLUSIONS

Arc, a growth factor and activity-regulated gene, codes for a cytoskeleton-associated protein, whose expression has been detected here after exposure to air nanoparticles for the first time. *Arc* relationship to *c-fos* oncogene, the member of *Ras* oncogene family, is important because it is associated with tumor growth. *Arc* belongs to a group of "immediate early expressed genes" specific for early brain changes. Some of these changes are predisposing to tumor growth, such as changes of the cytoskeleton. This particular gene, *Arc*, was found in this study to be upregulated in the brain after exposure to nanoparticles, in particular, to coarse nanoparticles. Our data are in line with previous results on the ability of coarse particles to induce proinflammatory cytokines in alveolar macrophages and cultured human bronchial epithelial cells [5].

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The authors are very grateful to Drs. Jean Ospital and Barry Wallerstein from AQMD Brain & Lung Tumor and Air Pollution Foundation, Diamond Bar, CA 91765 for their active support in data discussions and experimental approaches.