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Bondy, Stephen C

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Inhaled ultrafine particulate matter affects CNS inflammatory processes and may act via MAP kinase signaling pathways

M. T. Kleinman^a, J. Araujo^b, A. Nel^c, C. Sioutas^d, A. Campbell^e, P. Q. Cong^a, H. Li^a, and S. C. Bondy^{a,*}

^aDepartment of Community and Environmental Medicine, University of California, Irvine, Irvine, CA 92697, USA

^bDepartment of Cardiology, University of California, Los Angeles, CA 90095, USA

^cDepartment of Medicine-Nanomedicine, University of California, Los Angeles, CA 90095, USA

^dDepartment of Civil/Environmental Engineering, University of Southern California, Los Angeles, CA 90089, USA

^ePharmaceutical Sciences, Western University of Health Sciences, Pomona, California 91766, USA

Abstract

In addition to evidence that inhalation of ambient particulate matter (PM) can increase cardiopulmonary morbidity and mortality, the brain may also constitute a site adversely effected by the environmental presence of airborne particulate matter. We have examined the association between exposure to PM and adverse CNS effects in apolipoprotein E knockout (ApoE^{-/-}) mice exposed to two levels of concentrated ultrafine particulate matter in central Los Angeles. Mice were killed 24 hr after the last exposure and brain, liver, heart, lung and spleen tissues were collected and frozen for subsequent bioassays. There was clear evidence of aberrant immune activation in the brains of exposed animals as judged by a dose-related increase in nuclear translocation of two key transcription factors, NF- κ B and AP-1. These factors, are involved in the promotion of inflammation. Increased levels of glial fibrillary acidic protein (GFAP) were also found consequent to particulate inhalation suggesting that glial activation was taking place. In order to determine the mechanism by which these events occurred, levels of several MAP kinases involved in activation of these transcription factors were assayed by Western blotting. There were no significant changes in the proportion of active (phosphorylated) forms of ERK-1, I κ B and p38. However the fraction of JNK in the active form was significantly increased in animals receiving the lower concentration of concentrated ambient particle (CAPs). This suggests that the signaling pathway by which these transcription factors are activated involves the activation of JNK.

Keywords

Ultrafine particles; nanoparticles; brain; inflammation; transcription factors

* Correspondence to: Stephen C. Bondy, Ph. D., Department of Community and Environmental Medicine, University of California, Irvine, Irvine, CA 92697-1825, U. S. A. Phone: 949-824-8077, Email: scbondy@uci.edu.

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1. Introduction

Numerous epidemiological and toxicological studies demonstrate that exposure to ambient particulate matter (PM) is associated with increased cardiopulmonary morbidity and mortality. The brain is another potential target for adverse effects after inhalation of particulate matter. It is known that inhaled, nanosize particles can penetrate the lungs and be deposited in extrapulmonary tissues (Kreyling *et al.*, 2002; Oberdorster *et al.*, 2002). There is also evidence that inhaled particles can reach the brain, either by transport along the olfactory nerve or possibly by penetration of a blood brain barrier that is compromised by systemic effects of PM (Oberdorster *et al.*, 2002). The activities of signaling pathways that mediate inflammatory responses can be up-regulated in the brains of mice exposed to concentrated ambient particles (CAPs) derived from areas near primary emission sources (Campbell *et al.*, 2005). In addition biomarkers of oxidative stress and tissue injury in brain are observed at higher concentrations in mice after exposure to CAPs for as long as 2-weeks post-exposure (Campbell *et al.*, 2005). In this study we examined the association between exposure to PM and adverse CNS effects in apolipoprotein E knockout (ApoE^{-/-}) mice. This mutant develops hypercholesterolemia and has been used as a model for atherosclerosis (Colman *et al.*, 2006, Zadelaar *et al.*, 2007). The cardiovascular system of the ApoE^{-/-} has been previously found to be especially susceptible to CAPs (Sun *et al.*, 2005; Araujo *et al.*, 2006).

This model is also of utility in the study of age-related neurological disorders. ApoE can decrease microglial activity and TNF- α secretion thus attenuating inflammatory responses in the brain (Lynch *et al.*, 2001). Impairments in cognitive performance have been observed in aged apolipoprotein E (apoE)-deficient mice, and apoE epsilon 4 allele is a human risk factor in Alzheimer's disease (AD) (Law *et al.*, 2003). ApoE is also the major cholesterol transporter in the brain and human carriers of the e4 allele of apoE are at a higher risk of developing Alzheimer's disease (Zerbinatti and Bu, 2005). It is also known that the brain of the apoE null mouse is more susceptible to oxidative stress than is the wild type background strain mouse. In the hippocampus, the absence of apoE has a clear impact on the oxidant/antioxidant status. Endogenous level of thiobarbituric acid-reactive substances (TBARS) was found to be markedly elevated whereas level of alpha-tocopherol was decreased in APOE-deficient mice compared to wild type mice (Ramassamy *et al.*, 2000; Ramassamy *et al.*, 2001). This might be mediated via activation of glial cells. Compared to wild type mice, glial cells cultured from apoE knockout mice exhibit an enhanced production of several pro-inflammatory markers in response to treatment with amyloid- β and other activating stimuli (LaDu *et al.*, 2001). ApoE deficiency also accelerates the age-related decline in efficacy of the blood brain barrier (Hafezi-Moghadam *et al.*, 2007). Thus the ApoE deficiency found in the ApoE^{-/-} mutant may render an animal susceptible to cerebral inflammation, especially since this mutant exhibits chronic systemic inflammation (Grainger *et al.*, 2004).

2. Materials and methods

2.1 Animals and exposure conditions

C57BL/6J 6-week-old male ApoE null mice were obtained from Jackson Laboratory (Bar Harbor, ME), fed a normal chow diet. Food and water were administered *ad libitum*. All mice were initially housed in the UCLA campus animal facility under specific pathogen-free conditions. Mice were transported to a mobile laboratory located in the downtown of Los Angeles, close to interstate freeway I-10 (~300 m). In the mobile laboratory, mice were housed in a Hazelton chamber (Brown and Moss, 1981) provided with a filter that excludes >99% of PM greater than 0.3 μ m.

Ambient air for the exposures was drawn into a particle concentrator (Kim *et al.*, 2001a; Kim *et al.*, 2001b) via a 2 m long and 7.5 cm diameter aluminum duct that minimized particle losses

due to electrostatic deposition. The inlet to the duct was about 2.2 m above the ground. The concentrated aerosols from the particle concentrator were delivered to whole-body animal exposure chambers (Oldham *et al.*, 2004; Kleinman *et al.*, 2005). Each exposure chamber was a sealed unit, sectioned for housing 18 mice per chamber. Temperature and airflow were controlled during the exposures to ensure adequate ventilation, minimize buildup of animal-generated contaminants (dander, ammonia and CO₂) and to avoid thermal stresses.

Three groups of apoE null mice were exposed to purified air and 2 levels of CAPs. The high-level group was exposed for 5 hr per day, 3 days per week for 6 weeks to ultrafine particles concentrated by a factor of 15.4 ± 3.2 and is referred to as CAP₁₅. The mass concentration to which these mice were exposed was $114.2 \mu\text{g}/\text{m}^3$. A fraction of this aerosol was diluted with purified air to provide a low-level exposure to the second group of mice, equivalent to ambient particles that were concentrated by a factor of 4.1 ± 0.9 , (CAP₄). This had a mass concentration of $30.4 \mu\text{g}/\text{m}^3$.

A sample of the high-level aerosol was analyzed for composition. Organic and elemental carbon constituents represented more than 50% of the mass of the CAP (Table 1), indicating some influence of combustion emissions as the source of CAP at this site. Sulfate and nitrate represented about 11% of the aerosol suggesting the influence of gas to particle conversions of SO₂ and NO₂ from industrial and motor vehicle emissions. The CAPs were also enriched with Fe, Ca and Al and also contained lesser amounts of other trace metals (Table 1).

Control mice were exposed to air purified by passage through scrubbers containing permanganate-impregnated alumina spheres and activated carbon for oxidation and adsorption of organic vapors followed by a high efficiency particle filter for the same periods. Mice were euthanized 24 hr after the last exposure by i.p injection with pentobarbital (65mg/kg), and tissue was harvested and frozen for subsequent bioassays. All animals received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institute of Health (NIH publication 86-23 revised 1985) and all protocols were approved by the UCLA Institutional Animal Care and Use Committee.

2.2 Preparation of samples

Cytoplasmic and nuclear fractions were prepared using the method of Lahiri & Ge (2000). The brain tissue from each animal was weighed and homogenized in an ice-cold buffer (10 mM HEPES pH 7.9, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM DTT, 0.5 mM PMSF and 0.5% NP-40). The samples were incubated at 0° for 10 min in order to allow detergent-induced lysis of the nuclear membrane, and then centrifuged (1500×g) at 4°C for 1 min. The supernatant containing the cytoplasmic constituents was collected and protease inhibitor cocktail was added. The samples were aliquoted and stored at -80°C. The nuclear pellet was resuspended in a buffer composed of: 20 mM HEPES pH 7.9, 400 mM NaCl, 1 mM DTT, 1 mM EDTA, 1 mM EGTA and 1 mM PMSF. The samples were then centrifuged at 11,000×g for 5 min at 4°C. The supernatant that contained nuclear extract was aliquoted and stored at -80°C.

2.3 Electrophoretic Mobility Shift Assay

The extent of NF-κB and AP-1 activation was determined in the nuclear fraction of brain tissue using a protocol developed by Promega (Madison WI). The amount of protein in 2 μl of the nuclear extract was determined by the BCA protein assay kit (Pierce, Rockford, IL) and 50 μg of each sample, incubated with 32P-labeled oligonucleotides containing either the NF-κB or the AP-1 consensus sequence, was loaded onto a gel. A negative control containing no cell extract, as well as competitor reactions were included. The specific competitor contained unlabelled NF-κB or AP-1 consensus nucleotide while the nonspecific competitor contained

unlabelled SP-1 consensus oligonucleotide. The competitor reactions also contained 50 µg of nuclear fraction derived from AI-treated mouse brains. X-ray films were manually developed and the intensity of each band was measured and quantitated using the image analyzer, Eagle Eye, from Stratagene (San Diego, CA).

2.4 Western Blots

The levels of ERK, pERK, JNK, pJNK, p38, p-p38 and glial fibrillary acidic protein (GFAP) were determined using Western blotting. Protein content was determined using the BCA protein assay kit (Pierce, Rockford, IL). 25 µg of each sample was resolved on a SDS-10% PAGE and transferred onto a nitrocellulose membrane (Biorad, Hercules, CA). After blocking overnight in TBST (20 mM Tris-HCl, 150 mM NaCl and 0.1% Tween 20) containing 5% nonfat milk, membranes were washed 4x and incubated for 1h with primary antibodies (mouse monoclonal antibody against pERK (1:500); rabbit polyclonal antibody against ERK (1:1500); goat polyclonal antibody against pJNK (1:500); rabbit polyclonal antibody against JNK-1 (1:500); rabbit polyclonal antibody against p38(1:500); and rabbit polyclonal antibody against p-p38 (1:500), all purchased from Santa Cruz Biotechnology, Inc., Santa Cruz, California. After washing the membranes 4x with TBST, they were incubated with appropriate secondary HRP-conjugated antibodies (1:10,000). Bands were detected with ECL reagents (Amersham Pharmacia Biotech, Piscataway, NJ) following the manufacturer's protocol. An antibody against actin (mouse monoclonal, 1:1500 dilution) purchased from Chemicon International (Temecula, CA) was used to insure equal loading of protein for each sample. The intensity of specific bands was measured and quantitated using the image analyzer.

2.5 Statistical Analysis

The differences among these groups were tested using one-way analysis of variance followed by the Tukey test. Values were accepted as significant if $p < 0.05$ level using a two-tailed criterion.

3. Results

3.1 Activation of NF-κB and AP-1

In cortical tissue, levels of AP-1 that had been translocated to the cell nucleus and thus were in the activated form, were increased in mice exposed to CAPs. This response was dose-dependent in that it was greater in the group receiving the more concentrated (CAP₁₅) exposure than in the air-exposed or low concentration group (Fig. 1a). Cortical levels of activated NF-κB also were significantly increased only in the group of mice receiving the most concentrated CAP₁₅ (Fig. 1b).

3.2 Changes in GFAP levels

Increases in the levels of glial fibrillary acidic protein (GFAP) represent the activation of astrocytes. Treatment with the higher level of particulates (CAP₁₅) did not alter GFAP levels in cortex but the lower level of particulates (CAP₄) led to a 46% increase in GFAP (Fig. 2).

3.3 Proportion of MAP kinases in the activated form

In order to find the upstream events leading to activation of transcription factors by CAPs, the MAP kinases p38, JNK, ERK and IκB were assayed in both their basal and their phosphorylated forms. The proportion of a kinase that is phosphorylated can indicate the extent to which it is in its active state that is capable of triggering transcription factors. No significant changes in p38, ERK or IκB were apparent, either in terms of total levels of the kinase or in the fraction that was in the phosphorylated form (data not shown). However, the percentage of JNK that was activated was significantly elevated more than 3-fold in the mice exposed to the lower

concentration of CAP₄ but was not significantly altered in the mice exposed to the higher concentration of CAP₁₅ (Fig. 3).

4. Discussion

In an earlier report, we described an increase of levels of cytokines interleukin-1 alpha (IL-1 α) and tumor necrosis factor alpha (TNF- α) in the cortex of mice exposed to airborne particulate matter. (Campbell *et al.*, 2005). These cytokines are associated with inflammatory processes. The current report describes the upstream events which are likely to underlie such increases in markers of inflammation. Both transcription factors AP-1 and NF- κ B are likely to be involved and may be activated by phosphorylated JNK in the CNS (Zabluka *et al.*, 2003, Lotocki *et al.*, 2006, Barr *et al.*, 2007). It remains to be determined how particulate matter can initiate such signaling events. We observed greater increases in pJNK and GFAP in mice exposed to low levels of PM (25 μ g/m³) than in mice exposed at a higher level (125 μ g/m³). The reason for their biphasic dose-response relationship between PM concentration and reaction of both GFAP and pJNK is unknown. It is possible that low level PM exposures elicit inflammatory responses mediated by MAP kinase pathways and higher level exposures lead to cell death. Further histochemical study is needed to look for evidence of overt cell toxicity. However it is also possible that high level exposures activate other pathways involved in a negative feedback. The elevated level of GFAP in the animals exposed to the lower dose of PM, suggests that inflammatory responses provoked in the brain by exposure of mice to CAP may be mediated by activation of astroglia.

This study was performed on a genetic strain of mouse that is especially susceptible to inflammatory stimuli. It remains to be determined whether wild type mice may exhibit a parallel response to PM.

Indices of inflammation are known to be chronically elevated in the aged brain (David *et al.*, 1997; Streit *et al.*, 1999). In several age-related neurological diseases such as Alzheimer's disease, such inflammation is further increased (Mrak *et al.*, 1995; Styren *et al.*, 1998). In view of the association of inflammation with neurodegenerative processes, it is possible that extended exposure to airborne particulate matter may accelerate the progression of such disorders. Whether nanoparticles act directly on cell surface receptors in the brain such as Toll-like receptor-2 (TLR2), or whether systemic events mediate these effects, is the subject of our current research.

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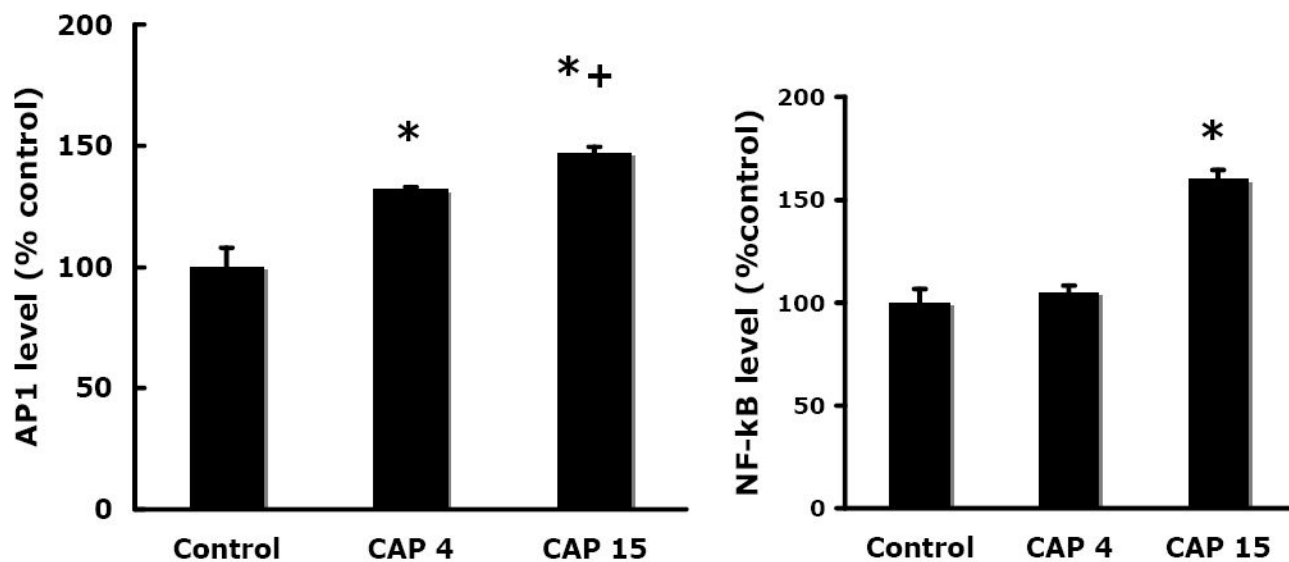
This work was supported in part by grants from the National Institutes of Health ES 7992 and AG 16794 to S.C. B.; US EPA STAR Awards RD83241301 to the Southern California Particle Center and RD 8319520 to M.K.; the National Institute of Environmental Health Sciences ES13432 to A.N.

References

- Araujo JA, Gong KW, Barajas B, Kleinman M, Harkema J, Sioutas C, Lusk AJ, Nel A. Ambient ultrafine particulate matter enhances atherosclerosis in apoE null animals. *Circulation* 2006;114:226–226. [PubMed: 16831986]
- Barr J, Sharma CS, Sarkar S, Wise K, Dong L, Periyakaruppan A, Ramesh GT. Nicotine induces oxidative stress and activates nuclear transcription factor kappa B in rat mesencephalic cells. *Mol Cell Biochem* 2007;297:93–99. [PubMed: 17021677]
- Brown MG, Moss OR. An inhalation exposure chamber designed for animal handling. *Lab Anim Sci* 1981;31:717–720. [PubMed: 7343770]
- Campbell A, Oldham M, Becaria A, Bondy SC, Meacher D, Sioutas C, Misra C, Mendez LB, Kleinman A. Particulate matter in polluted air may increase biomarkers of inflammation in mouse brain. *Neurotoxicology* 2005;26:133–140. [PubMed: 15527881]

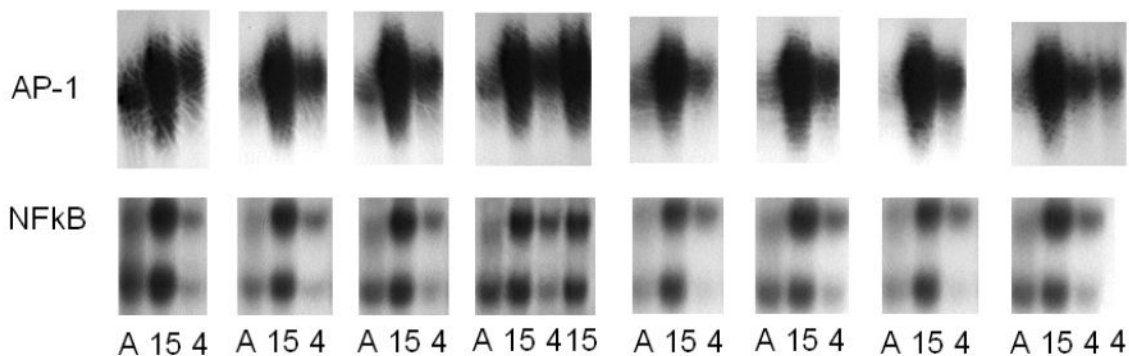
- Coleman R, Hayek T, Keidar S, Aviram M. A mouse model for human atherosclerosis: long-term histopathological study of lesion development in the aortic arch of apolipoprotein E-deficient (E0) mice. *Acta Histochem* 2006;108:415–424. [PubMed: 17007910]
- David JP, Ghazali F, Fallet-Bianco C, Watez A, Delaire S, Boniface B, Di Menza C, Delacourte A. Glial reaction in the hippocampal formation is highly concentrated with aging in human brain. *Neurosci Lett* 1997;235:53–56. [PubMed: 9389594]
- Grainger DJ, Reckless J, McKilligin E. Apolipoprotein E modulates clearance of apoptotic bodies in vivo and in vitro, resulting in a systemic proinflammatory state in apolipoprotein E-deficient mice. *J Immunol* 2004;173:6366–6375. [PubMed: 15528376]
- Hafezi-Moghadam A, Thomas KL, Wagner DD. ApoE deficiency leads to a progressive age-dependent blood-brain barrier leakage. *Am J Physiol Cell Physiol* 2007;292:C1256–C1262. [PubMed: 16870825]
- Kim S, Jaques PA, Chang MC, Barone T, Xiong C, Friedlander SK, Sioutas C. Versatile aerosol concentration enrichment system (VACES) for simultaneous in vivo and in vitro evaluation of toxic effects of ultrafine, fine and coarse ambient particles - Part II: Field evaluation. *J Aerosol Sci* 2001a; 32:1299–1314.
- Kim S, Jaques PA, Chang MC, Froines JR, Sioutas C. Versatile aerosol concentration enrichment system (VACES) for simultaneous in vivo and in vitro evaluation of toxic effects of ultrafine, fine and coarse ambient particles - Part I: Development and laboratory characterization. *J Aerosol Sci* 2001b; 32:1281–1297.
- Kleinman MT, Hamade A, Meacher D, Oldham M, Sioutas C, Chakrabarti B, Stram D, Froines JR, Cho AK. Inhalation of concentrated ambient particulate matter near a heavily trafficked road stimulates antigen-induced airway responses in mice. *J Air Waste Manag Assoc* 2005;55:1277–1288. [PubMed: 16259423]
- Kreyling WG, Semmler M, Erbe F, Mayer P, Takenaka S, Schulz H, Oberdorster G, Ziesenis A. Translocation of ultrafine insoluble iridium particles from lung epithelium to extrapulmonary organs is size dependent but very low. *J Toxicol Environ Health A* 2002;65:1513–1530. [PubMed: 12396866]
- LaDu MJ, et al. Apolipoprotein E and apolipoprotein E receptors modulate A beta-induced glial neuroinflammatory responses. *Neurochem Int* 2001;39:427–434. [PubMed: 11578778]
- Lahiri DK, Ge Y. Electrophoretic mobility shift assay for the detection of specific DNA-protein complex in nuclear extracts from the cultured cells and frozen autopsy human brain tissue. *Brain Research Protocols* 2000;5:257–265. [PubMed: 10906491]
- Law A, Gauthier S, Quirion R. Alteration of nitric oxide synthase activity in young and aged apolipoprotein E-deficient mice. *Neurobiol Aging* 2003;24:187–190. [PubMed: 12493565]
- Lotocki G, de Rivero Vaccari JP, Perez ER, Alonso OF, Curbelo K, Keane RW, Dietrich WD. Therapeutic hypothermia modulates TNFR1 signaling in the traumatized brain via early transient activation of the JNK pathway and suppression of XIAP cleavage. *Eur J Neurosci* 2006;24:2283–2290. [PubMed: 17074049]
- Lynch JR, Morgan D, Mance J, Matthew WD, Maskowitz DT. Apolipoprotein E modulates glial activation and the endogenous cerebral inflammatory response. *J Neuroimmunol* 2001;114:107–113. [PubMed: 11240021]
- Mrak RE, Sheng JG, Griffin WST. Glial cytokines in Alzheimer's Disease: review and pathogenic implications. *Human Pathol* 1995;26:816–823. [PubMed: 7635444]
- Oberdorster G, Sharp Z, Atudorei V, Elder A, Gelein R, Lunts A, Kreyling W, Cox C. Extrapulmonary translocation of ultrafine carbon particles following whole-body inhalation exposure of rats. *J Toxicol Environ Health A* 2002;65:1531–1543. [PubMed: 12396867]
- Oldham MJ, Phalen RF, Robinson RJ, Kleinman MT. Performance of a portable whole-body mouse exposure system. *Inhal Toxicol* 2004;16:657–662. [PubMed: 16036757]
- Ramassamy C, Averill D, Beffert U, Theroux L, Lussier-Cacan S, Cohn JS, Christen Y, Schoofs A, Davignon J, Poirier J. Oxidative insults are associated with apolipoprotein E genotype in Alzheimer's disease brain. *Neurobiol Dis* 2000;7:23–37. [PubMed: 10671320]
- Ramassamy C, Krzywkowski P, Averill D, Lussier-Cacan S, Theroux L, Christen Y, Davignon J, Poirier J. Impact of apoE deficiency on oxidative insults and antioxidant levels in the brain. *Brain Res Mol Brain Res* 2001;86:76–83. [PubMed: 11165374]

- Streit WJ, Walter SA, Pennell NA. Reactive microgliosis. *Prog Neurobiol* 1999;57:563–581. [PubMed: 10221782]
- Styren SD, Kamboh MI, Dekosky ST. Expression of differential immune factors in temporal cortex and cerebellum: the role of α -1-antichymotrypsin, Apolipoprotein E, and reactive glia in the progression of Alzheimer's Disease. *J Comp Neurol* 1998;396:511–520. [PubMed: 9651008]
- Sun Q, Wang A, Jin X, Natanzon A, Duquaine D, Brook RD, Aguinaldo JG, Fayad ZA, Fuster V, Lippmann M, Chen LC, Rajagopalan S. Long-term air pollution exposure and acceleration of atherosclerosis and vascular inflammation in an animal model. *Jama* 2005;294:3003–3010. [PubMed: 16414948]
- Zablocka B, Dluzniewska J, Zajac H, Domanska-Janik K. Opposite reaction of ERK and JNK in ischemia vulnerable and resistant regions of hippocampus: involvement of mitochondria. *Brain Res Mol Brain Res* 2003;110:245–252. [PubMed: 12591160]
- Zadelaar S, Kleemann R, Verschuren L, de Vries-Van der Weij J, van der Hoorn J, Princen HM, Kooistra T. Mouse models for atherosclerosis and pharmaceutical modifiers. *Arterioscler Thromb Vasc Biol* 2007;27:1706–1721. [PubMed: 17541027]
- Zerbinatti CV, Bu G. LRP and Alzheimer's disease. *Rev Neurosci* 2005;16:123–135. [PubMed: 15959937]



1a

1b



Note: A=Control, 15=CAP 15, 4= CAP 4

Figure 1. Cortical levels of a) AP-1 and b) NF-κB following inhalation of CAPs
 Integrated density of the shifted AP-1 bands in cortex relative to control value. CAP4 = 4-fold concentrated particulates, CAP15 = 15-fold concentrated particulates. * Value differs ($P \leq 0.05$) from control group receiving purified air, + Value differs ($P \leq 0.05$) from both other groups. Bars represent mean of 6-7 samples \pm S.E. Photographs of each gel shift analyzed are also shown.

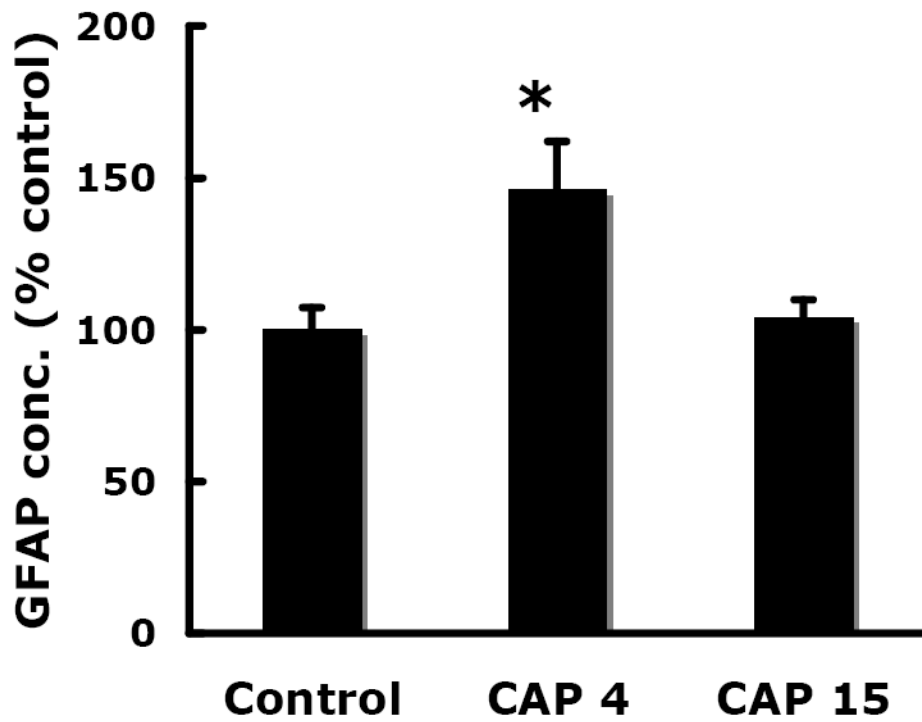


Figure 2. Levels of GFAP in cortex of animals exposed to CAPs

Integrated density of the GFAP bands derived from Western blotting. Bars represent mean of 6 samples \pm S.E. relative to control value. CAP4 = 4-fold concentrated particulates, CAP15 = 15-fold concentrated particulates. * Value differs ($P \leq 0.05$) from group receiving purified air.

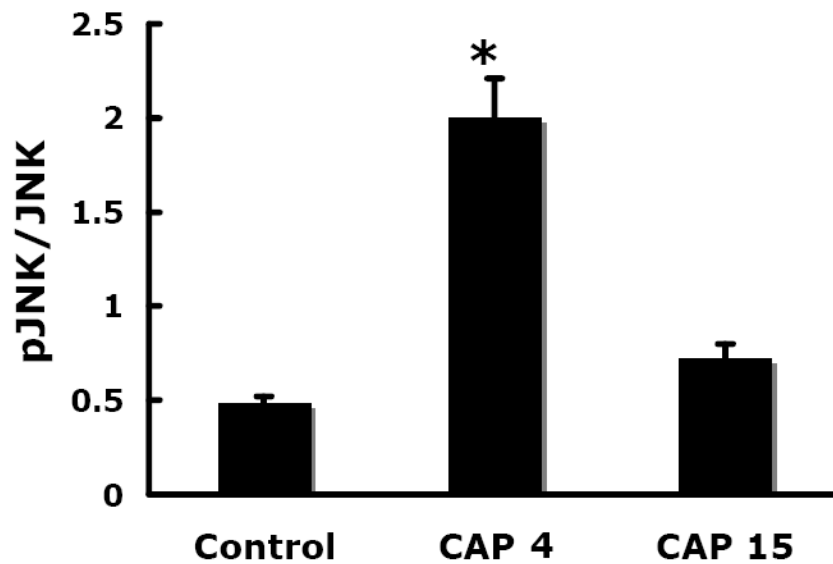


Figure 3. Proportion of cortical JNK in activated form following exposure to CAPs
Integrated density of the bands derived from Western blotting. CAP4 = 4-fold concentrated particulates, CAP15 = 15-fold concentrated particulates. Value expressed as ratio of phosphorylated pJNK to that for JNK. *: value is significantly different ($P \leq 0.05$) from the group receiving purified air. Bars represent mean of 6 samples \pm S.E. Since the sensitivity of antibodies for pJNK and JNK are likely to differ, this ratio is not absolute but changes represent a relative shift in the proportions of the two forms of JNK.

Table 1**Composition of concentrated ultrafine particles (CAP₁₅)**Values are mean of 2-3 assays of a single composite sample and are given as $\mu\text{g}/\text{m}^3$

Total Mass	114.2
Organic Carbon	54.0
Elemental carbon	8.9
Chloride	0.7
Nitrate	4.1
Phosphate	1.7
Sulfate	8.6
Sodium	0.1
Ammonium	3.2
Potassium	0.2
Other metallic elements	6.6