Carbon Black Decreases Cellular Proliferation and Alters Mitochondrial Morphology

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ABSTRACT

Nanoparticles of pure carbon, carbon black (CB), are a common atmospheric pollutant. In industrialized and heavily populated areas. It is produced primarily via combustion of fossil fuels and represents a significant health hazard. It is known to worsen asthma and bronchitis when inhaled and to cause inflammation, heart dysfunctions, and oxidative stress when incorporated into other organs. The key focal point of this work is to examine markers of stress signaling and cellular dysfunction when human bronchial epithelial cells (16HBE14+) are exposed to CB particles ranging upward in size from 70 nm and averaging 130 nm in diameter. BrdU incorporation studies revealed CB exposure to reduce rates of cell division. Additionally, cells were shown to induce apoptosis at significantly higher rates. Within 12 hours of CB exposure, reactive oxygen species (ROS) were shown to rise significantly, a clear marker of oxidative stress. Chronic exposure (24 days) of cells to low doses of CB revealed a clear impact upon cell division and survival. Sirtuin 1, a stress regulated protein deacetylase in the cytosol, was assessed for stability following CB exposure. No significant changes were observed in the stability of Sirtuin 1, which is known to be a stress inducible mitochondrial protein. When using microscopy to evaluate cellular morphological changes in response to CB exposure, mitochondria were shown to display abnormal morphology. HK2 cells were treated with varying doses of CB, fixed, and prepared for immunocytochemistry of the mitochondria (Tom20-Alexa488, mitochondria (GAPDH-Alexa594), and the nucleus (DAPI). Using software developed in MatLab, mitochondria were analyzed for changes in mitochondrial size and localization. Significant changes were identified with regard to an increase in mitochondrial size, and strong trends were observed in an increased localization preference for the peripheral region. A discussion of the link between elevated ROS levels and mitochondrial behavior will be discussed.

RESULTS

Figure 2: Atomic Force Microscopy of Carbon Black Nanoparticles. AFM was used to image CB nanoparticles and size was analyzed using Gwyddion software. Average size is 358.03 nm with a standard deviation of 22.61 nm (n=100). BrdU was added to the cells 24 hours prior to fixation with 4.4% PFA after 24 hours. Analysis was performed using software developed by McClatchey et al. at University of Colorado (b).

Figure 3: Probe sonication techniques distribute carbon black in solution on the nanoparticle scale as tested by Micrometrics Analytical Services (Norcross, GA). A mixture of particles at 1 mg/mL bovine serum albumin (BSA) in PBS at 6.5 mg/mL, and distilled water was sonicated for 5 minutes at a frequency of 500 W ultrasonic probe. CB particles exhibited a range of diameters from 70 - 660 nm with an average size of 220 nm.

Figure 4: Chronic low dose CB exposure decreases cellular viability as treatment duration increases. A) Healthy v. CB exposed cells at 10 μg/mL CB. At each passage, 20% of cells were passaged and 70% of the cytoplasm furthest from the nearest nucleus (% of the cytoplasm furthest from the nearest nucleus) increased in perinuclear preference (10%) and retained picnotic volume loss decreased. Cell density for each treatment and time point were normalized to mock treatment. Values represented with *SE.

Figure 5: Carbon black reduces levels of proliferation at 24 hours. Cellular proliferation was determined through 8 hours of BrdU incorporation followed by fixation at 24 hours post CB poisoning. A) Side-by-side comparison of total nuclear (DAP) and BrdU incorporated nuclei for control and 150 μg/mL CB treatments. B) Percentage representation of BrdU incorporation. Cells were treated with 0, 5, 10, 20, 50, and 150 μg/mL CB. At each fixation, 20% of cells were passaged and 70% of the cytoplasm furthest from the nearest nucleus (10%) and retained picnotic volume loss decreased. Cell density for each treatment and time point were normalized to mock treatment. Values represented with *SE.

Figure 6: Mitochondrial imaging for imaging assays, HCE cells were seeded at 25,000 cells/cm² on glass cover slips in 24-well plates and treated with low dose CB, 150μg/mL, and 250μg/mL. Cells were fixed with 0.5%, 4.4% PFA after 24 hours. For fluorescence imaging, all cover slips were sealed in 200 μL of 0.5% BSA solution and followed by 300G of primary antibody solution (primary antibody stock solution, 1:1000 dilution Phalloidin, and 1:150 dilution Tom20). Coverslips were mounted onto microscope slides in Prolong Gold Antifade with DAPI. One slide per treatment for each replicate was imaged, and 10 pictures of each coverlip were captured using a Leica TII Eclipse microscope at 10X. Analysis was performed using software developed by McClatchey et al. at University of Colorado (b).

Figure 7: CB induces morphological changes in lung cell mitochondria. Average particular preference and average mitochondrial length and width were measured using software developed by McClatchey et al. at University of Colorado (b). Average mitochondrial measurements for each treatment were compared to a control. A increasing trend in mitochondrial perinuclear preference was observed in cells exposed to higher dosages of CB compared to mock cells (a). Perinuclear preference is defined by McClatchey et al. as the natural log of the ratio of the mitochondrial mass in the 30% of the cytoplasm closest to the nearest nuclear mass of the mitochondria in the 30% of the cytoplasm furthest from the nearest nuclear mass (b). Additionally, a slight increasing trend in mitochondrial length was seen as CB exposure increased (b). A slight increasing trend in average mitochondrial width was also observed at higher dosages of CB (c). No consistent trend was noted in the average fraction of the cytoplasm filled by mitochondria between treatments (d). *Denotes significance of p < 0.05 using a paired t-test. Values represented with *SE.

LITERATURE CITED

- CB exposure decreases rates of proliferation at 24 hours.
- Chronic exposure to low dose CB reduces number of cells as duration of exposure increases.
- CB induces a trending increase in perinuclear preference.
- CB causes a significant increase in average mitochondrial length.
- CB induces a slight increasing trend in mitochondrial width.
- CB does not alter the fraction of the cytoplasm filled by mitochondria.

CONCLUSIONS

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