MicroRNAs are Involved in DNA Damage and Cytotoxicity in Human Bronchial Epithelial Cells Concurrently Exposed to Ambient Particulate Matter and Ethanol

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Abstract

Heavy ethanol consumption is associated with an increased risk for many types of cancers, such as colorectal, liver, esophageal, and throat cancers. Ethanol is considered as a co-carcinogen: it promotes tumorigenesis in the presence of other carcinogenic agents. Outdoor air pollution contains a mixture of chemicals and toxins that have been associated with an increased risk for lung cancer. However, there are no studies investigating their combined effects. **We hypothesized that co-exposure to urban particulate matter (U-PM) and ethanol may lead to enhanced lung injury by inducing DNA damage, suppressing DNA damage response, and enhancing cytotoxicity, and that microRNAs are involved in DNA damage and cytotoxicity induced by concurrent exposure to U-PM and ethanol.** Our results showed that exposure of cells to 100 μg/mL and lower doses of U-PM, or 100 mM and lower doses of ethanol did not increase cytotoxicity. However, co-exposure of cells to non-cytotoxic doses of U-PM and ethanol resulted in enhanced cytotoxicity. We also found that exposure to U-PM increased the expression of phosphorylated H2AX, a marker for DNA damage, which can be enhanced by concurrent exposure to ethanol. While exposure of cells to non-cytotoxic doses of U-PM alone increased ROS generation, co-exposure to U-PM and ethanol at non-cytotoxic doses did not enhance U-PM-induced ROS generation. We also found that exposure to U-PM and/or ethanol resulted in the upregulation of miR-153, miR-155, and miR-210 in human bronchial epithelial cells. These results suggest that co-exposure to U-PM and ethanol may enhance lung injury. The findings from this study may have important implications for understanding the cytotoxic effects of concurrent exposure to U-PM and ethanol and the potential roles of microRNAs in mediating DNA damage and cytotoxicity in human bronchial epithelial cells concurrently exposed to U-PM and ethanol.

Background

Lung Cancer

- The leading cause of cancer deaths in the US
- 2018: About 234,030 new cases of lung cancer, about 154,050 deaths from lung cancer
- Risk factors: (1) Smoking: ~ 80%; (2) Outdoor air pollution and others: ~ 20%
- Outdoor air pollution is the leading environmental cause of cancer deaths.
- In 2013, IARC classified outdoor air pollution, including particulate matter (PM), as carcinogenic to humans (Group 1).

Particulate Matter (PM)

- "A complex mixture of extremely small particles and liquid droplets, including acids, organic chemicals, metals, and soil or dust particles." (United States EPA)
- Many epidemiological studies have shown a linkage between PM pollution and increased respiratory and cardiovascular morbidity and mortality.
- Several epidemiological studies have shown that long-term exposure to ambient PM is an important and independent environmental risk factor for lung cancer.

PM, Oxidative Stress and DNA Damage

- Exposure to PM caused ROS generation *in vivo* and *in vitro*. Through the ROS-mediated reaction, PM may cause direct or indirect DNA damage, lipid peroxidation and protein modification.
- ROS can mimic or interfere with secondary messengers in cell signaling pathways.
- DNA damage will induce DNA damage response to initiate including phosphorylation of H2AX (γ-H2AX).

Ethanol

- Ethanol exposure is linked with colorectal, liver, esophageal, and throat cancers in a dose-dependent manner.
- Acetaldehyde, a metabolite of ethanol, can interfere with DNA replication, induce DNA damage, and form adducts.
- Cytochrome P450 2E1 (CYP2E1) is an alternative pathway of ethanol oxidation into acetaldehyde that also produces ROS.
- Ethanol is considered a co-carcinogen: cancer development is promoted in the presence of other carcinogens.
- Ethanol abuse may lead to alcoholic lung disease and increases the risk of developing acute respiratory distress syndrome.

H2A histone family, member X (H2AX)

- Histones are the chief protein components of chromatin, acting as spools around which DNA winds, and they play a role in gene regulation.
- They are highly conserved and can be grouped into five major classes: H1/H5, H2A, H2B, H3, and H4. The H2AX contributes to the histone-formation and therefore the structure of DNA.
- H2AX undergoes phosphorylation on Serine 139, called γH2AX, as a reaction on DNA Double-Strand Breaks (DSBs).
- γH2AX is a sensitive target for probing DSBs in cells.

Materials and Methods

Urban Particulate Matter SRM 1648a (U-PM)

- Purchased from the National Institute of Standards and Technology (NIST, Gaithersburg, MD)
- Prepared from urban PM collected in the St. Louis, MO, area in a baghouse
- U-PM particles have a mean diameter of 5.85 μm, and consist of greater than 63% inorganic carbon and 4–7% organic carbon.
- Other major constituent elements include Al, Fe and K, and minor constituents include Na, Pb and Zn.

Human Lung Bronchial Epithelial Cells (BEAS-2B)

- The major cellular targets of inhaled particles.
- Commonly used to investigate the mechanisms of lung carcinogenesis induced by various chemicals, such as in metal-induced carcinogenesis.
- Obtained from American Type Culture Collection (ATCC) (Rockville, MD).

Methods

- Cytotoxicity: alamarBlue assay (Invitrogen).
- Reactive oxygen species (ROS) generation: 2', 7'-dichlorodihydrofluorescein diacetate (H_2DCF-DA). DCF fluorescence was measured by microplate reader (Synergy HT).
- Western blot: γ-H2AX protein expression.
- miR-153, 155, and 210 expression: mirVana miRNA Isolation Kit and TaqMan MicroRNA Assays (Applied Biosystems).

Results

Effects of exposure to U-PM or ethanol alone on cytotoxicity in human bronchial epithelial cells

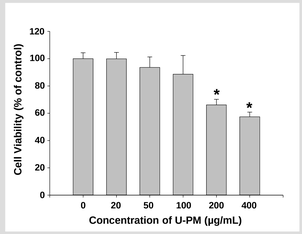

Fig.1. Cytotoxicity in BEAS-2B cells exposed to U-PM or ethanol alone. 7x10^3 cells were seeded in each well of 96-well plates. After overnight culture, cells were treated with U-PM or ethanol alone for 24h. Cells without any treatment were used as control. Cytotoxicity was determined by alamarBlue assay (Invitrogen). Data are shown as mean ± SD (n=6). *, p<0.01 vs. Control.

Concurrent exposure to U-PM and ethanol increased cytotoxicity in human bronchial epithelial cells

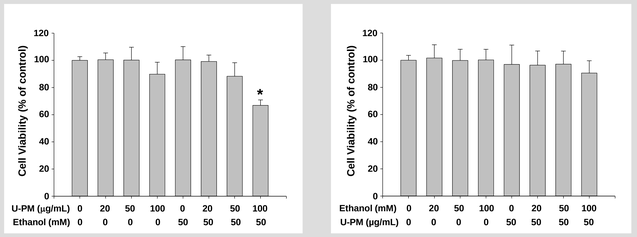

Fig. 2. Cytotoxicity in BEAS-2B cells concurrently exposed to U-PM and ethanol. 7x10^3 cells were seeded in each well of 96-well plates. After overnight culture, cells were treated with U-PM and/or ethanol for 24h. Cells without any treatment were used as control. Cytotoxicity was determined by alamarBlue assay (Invitrogen). Data are shown as mean ± SD (n=6). *, p<0.01 vs. Control.

Effects of concurrent exposure to U-PM and ethanol on ROS generation in human bronchial epithelial cells

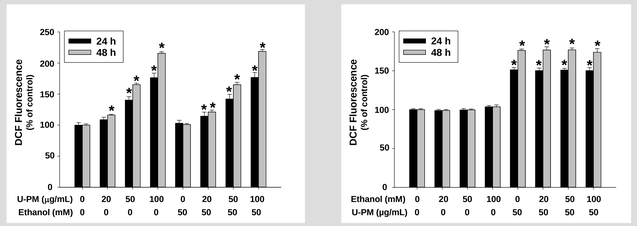

Fig. 3. ROS generation in BEAS-2B cells exposed to U-PM and/or ethanol. 10^4 cells were seeded in each well of 96-well plates. After overnight culture, cells were pretreated with 5 μM of H_2-DCFDA for 2h, followed by treatment with U-PM and/or ethanol for 24 and 48h. Cells without any treatment were used as control. Data are shown as mean ± SD (n=6). *, p<0.01 vs. Control.

Ethanol exposure enhanced U-PM-induced up-regulation of γ-H2AX in human bronchial epithelial cells

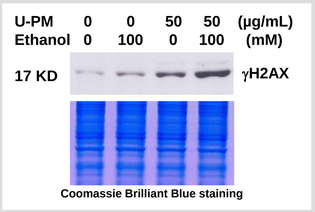

Fig. 4. γ-H2AX expression in BEAS-2B cells exposed to U-PM and/or ethanol. BEAS-2B cells were treated with U-PM and/or ethanol for 24h. Cells without treatment were used as control. Nuclear protein was extracted by NE-PER Nuclear and Cytoplasmic Extraction Reagent (PIERCE) and subjected to Western blot. Coomassie Brilliant Blue stained gel served as loading control.

Concurrent exposure to U-PM and ethanol altered microRNA expression in human bronchial epithelial cells

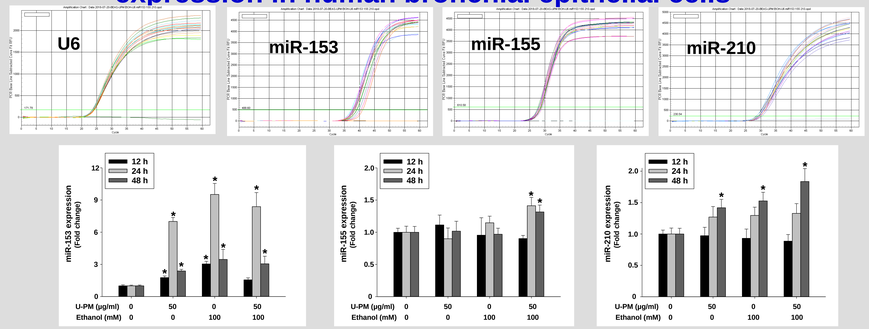

Fig. 5. miRNA expression in BEAS-2B cell exposed to U-PM and/or ethanol. 2x10^5 cells were seeded in each well of 6-well plates. After overnight culture, cells were treated with U-PM and/or ethanol for 12, 24, and 48h. miRNA expression was analyzed by using mirVana miRNA Isolation Kit and TaqMan MicroRNA Assays. Values of miRNA expression were normalized to the endogenous control U6, and reported as fold change as compared to those in the controls. Data are shown as mean ± SD (n=3). *, p<0.05 vs. Control.

Conclusions

Conclusions

- Exposure of human bronchial epithelial cells to 100 μg/mL and lower doses of U-PM, and 100 mM and lower doses of ethanol did not cause cytotoxicity. However, exposure to ≥ 200 μg/mL of U-PM and ≥ 200 mM of ethanol caused significant cytotoxicity.
- Co-exposure of cells to non-cytotoxic doses of U-PM and ethanol resulted in enhanced cytotoxicity and DNA damage in human bronchial epithelial cells.
- Exposure of cells to non-cytotoxic doses of U-PM alone increased ROS generation. However, exposure to non-cytotoxic doses of ethanol did not increase ROS generation. Co-exposure to U-PM and ethanol at non-cytotoxic doses did not enhance U-PM-induced ROS generation.
- Exposure of cells to U-PM and/or ethanol resulted in upregulation of miR-153, miR-155, and miR-210 in human bronchial epithelial cells.
- These findings may have important implications on understanding the cytotoxic effects of concurrent exposure to U-PM and ethanol and the potential roles of microRNA in mediating DNA damage and cytotoxicity in human bronchial epithelial cells concurrently exposed to PM and ethanol.

Future Directions

- Investigation of long-term effects of concurrent exposure to U-PM and ethanol on DNA damage and cytotoxicity in human bronchial epithelial cells and in animal models would be an important future direction.
- Elucidation of the detailed mechanisms by which microRNAs modulate DNA damage and cytotoxicity in human bronchial epithelial cells concurrently exposed to U-PM and ethanol, including the identification of the direct targets of miR-153, miR-155 and miR-210 and the investigation of their potential roles in modulating DNA damage and cytotoxicity.

Acknowledgement

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