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DNA damage caused by inorganic particulate matter on Raji and HepG2 cell lines exposed to ultraviolet radiation

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ABSTRACT

Epidemiological studies have correlated exposure to ultraviolet-irradiated particulate matter with cardiovascular, respiratory, and lung diseases. This study investigated the DNA damage induced by two major inorganic particulate matter compounds found in diesel exhaust, ammonium nitrate and ammonium sulfate, on Burkitt's lymphoma (Raji) and hepatocellular carcinoma (HepG2) cell lines. We found a dose-dependent positive correlation of accumulated DNA damage at concentrations of ammonium nitrate (25 μg/ml, 50 μg/ml, 100 μg/ml, 200 μg/ml, 400 μg/ml) with ultraviolet exposure (250 J/m^2, 400 J/m^2, 600 J/m^2, 850 J/m^2), as measured by the comet assay in both cell lines. There was a significant difference between the treated ammonium nitrate samples and negative control samples in Raji and HepG2 cells ($p < 0.001$). Apoptosis was shown in Raji and HepG2 cells when exposed to high concentrations of ammonium nitrate (200 μg/ml and 400 μg/ml) for 1 h in samples without ultraviolet exposure, as assessed by the comet assay. However, the level of apoptosis greatly diminished after ultraviolet exposure at these concentrations. Over a 24 h period, at intervals of 1, 4, 8, 12, 18, and 24 h, we also observed that ammonium nitrate decreased viability in Raji and HepG2 cell lines and inhibited cell growth. Ammonium sulfate-induced DNA damage was minimal in both cell lines, but there remained a significant difference ($p < 0.05$) between the ultraviolet radiation treated and negative control samples. These results indicate that the inorganic particulate compound, ammonium nitrate, induced DNA strand breaks at all concentrations, and indications of apoptosis at high concentrations in Raji and HepG2 cells, with ultraviolet radiation preventing apoptosis at high concentrations. We hypothesize that ultraviolet radiation may inhibit an essential cellular mechanism, possibly involving p53, thereby explaining this phenomenon. Further studies are necessary to characterize the roles of apoptosis inhibition induced by DNA damage caused by inorganic particulate matter.

1. Introduction

Epidemiologically, particulate matter (PM) has been shown to cause a variety of harmful human health effects, such as increased mortality from both short term and long term exposure [1–3]. Short term exposure to PM has been shown to increase respiratory and heart disease occurrences [4,5]. Long term effects include the development of lung cancer and cardiovascular diseases in patients exposed to PM [6]. Recent studies also show that PM may be able to enter and affect more obscure areas of the body, such as the bloodstream and the liver [7,8]. PM exposure accounts for approximately 500,000 deaths worldwide every year [9]. In air pollution, PM involves a complex mixture of chemicals including polycyclic aromatic hydrocarbons (PAHs), sulfates, nitrates, and other compounds [10]. The mechanisms by which PM induces harmful health effects have been shown to include the generation of reactive oxygen species, oxidative stress, and inflammation [11–13].

PM in the atmosphere consists of two major inorganic pollutants deleterious to human health and found in diesel exhaust, nitrates and sulfates [14]. Ultraviolet radiation has been shown to be a leading cause of skin cancer, skin aging, and genetic mutations in peripheral blood erythrocytes, bone marrow, and the lung [15–17]. In addition, ultraviolet radiation has also been shown to increase

Abbreviations: PM, particulate matter; PAHs, polycyclic aromatic hydrocarbons.

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DNA damage in cells exposed to particulates or their chemical derivatives, such as titanium dioxide, through oxidative pathways. This effect has also been confirmed with organic compounds such as polycyclic aromatic hydrocarbons [18–23]. However, the effects of ultraviolet radiation on the genotoxic effects of nitrates and sulfates have not been studied extensively [24]. In addition, the effects of inorganic pollutants in PM (such as nitrates and sulfates) on the bloodstream and liver have not been previously studied, although recent studies show that organic PM may cause deleterious effects in these regions [7]. The purpose of this study was to determine the DNA damage and possible mutagenicity of ultraviolet radiation on two cell lines, Burkitt's lymphoma (Raji) and hepatocellular carcinoma (HepG2), when exposed to ammonium nitrate and ammonium sulfate.

The alkaline comet assay is a sensitive *in vitro* method for accurately determining DNA damage, including single and double strand breaks, alkali labile sites, and oxidative stress [25–31]. The method has been used to detect DNA damage caused by a large variety of compounds in Raji and HepG2 cells [32–37]. In the procedure, negatively charged chromatin fragments from a single cell are allowed to migrate through an electrified current to the anode. By this principle, shorter DNA fragments migrate faster than longer DNA fragments, causing the formation of a "comet", as seen under the epifluorescence microscope. The extent of DNA damage determines the nature of the comet (tail profile, shape, *etc.* [38]). The comet assay was used in this study to investigate the genotoxic effects of ultraviolet radiation on Raji and HepG2 cells exposed to ammonium nitrate and ammonium sulfate. Trypan blue exclusion was also used as a suitable assay to assess cell viability in Raji and HepG2 cells [39,40].

2. Materials and methods

2.1. Chemicals and reagents

Low melting point agarose (LMPA) was purchased from Promega (Madison, WI). Ammonium nitrate was obtained from Flinn Scientific (Batavia, IL). All other chemical reagents used were supplied by Sigma Chemical Company (St. Louis, MO).

2.2. Cell culture

Raji and HepG2 cells were obtained from the American Type Culture Collection (ATCC; Manassas, VA) and cultured in RPMI 1640 medium supplemented with 10% Fetal Bovine Serum (FBS, Hyclone Laboratories, Inc., Logan, UT). Cells were incubated at 37 °C with 5% CO_2. Media was replaced 2 times per week. Cells used for experimentation were in exponential growth phase and of at least 90% viability, as determined by trypan blue exclusion.

2.3. Alkaline comet assay

2.3.1. Exposure

Raji and HepG2 cells were exposed to ammonium nitrate and ammonium sulfate at concentrations of 25 μg/ml, 50 μg/ml, 100 μg/ml, 200 μg/ml, and 400 μg/ml for 1 h at room temperature. The cells were subsequently exposed to ultraviolet radiation with a UV-Crosslinking Spectrolinker (Spectronics Corporation, Westbury, NY) at intensities of 250 J/m^2, 400 J/m^2, 600 J/m^2, and 850 J/m^2. After ultraviolet exposure, cells were placed at 4 °C for 20 min. Negative controls were established for ammonium nitrate, ammonium sulfate, and ultraviolet radiation samples.

2.3.2. Procedure

The protocol of Fairbairn et al. [28] was followed with slight modifications. Raji and HepG2 cell suspensions exposed to ammonium nitrate or ammonium sulfate and irradiated with ultraviolet radiation were mixed with 1.25% low-melting point agarose (1.25% LMPA Agarose, 1 × PBS) in a 1:1 ratio for a final concentration of 0.75% and pipetted onto custom-made frosted slides with clear windows in the center of the slides. Electrophoresis took place at 24 V and 400 mA for 20 min. The slides were stained with propidium iodide at a concentration of 2.5 μg/ml for 20 min and analyzed by epifluorescence microscopy (Zeiss Axioscope) at 40× magnification. A minimum of 50 comets per sample were scored with open source image analysis software (OpenComet v1.2). As suggested by Collins et al. and Lovell et al. [41,42], the DNA percentage in the tail (% DNA Tail) and Olive Tail Moment were used as parameters for quantifying DNA damage [26,42]. A minimum of five independent experiments were performed for each ammonium nitrate or ammonium sulfate concentration and ultraviolet radiation intensity.

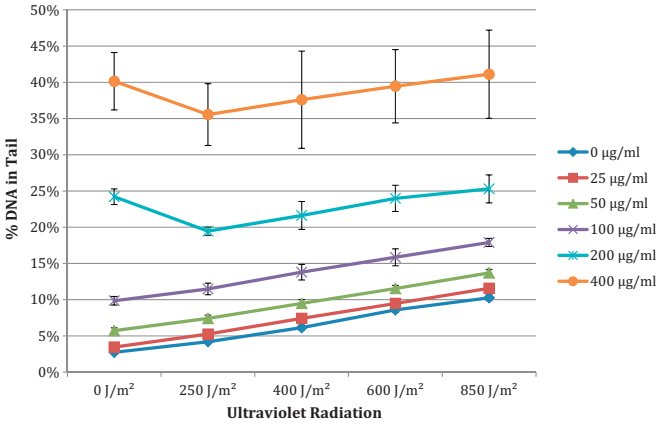

Fig. 1. (Raji ammonium nitrate): DNA damage induced by ammonium nitrate and ultraviolet radiation in Raji cells, as assessed by the comet assay ($p < 0.001$). All values are represented as mean ± standard error of mean. The "% DNA in Tail" parameter represents the accumulated DNA damage in the tails of the comets observed under the epifluorescence microscope.

2.4. 24 h Incubation viability and cell proliferation analysis

2.4.1. Viability analysis

Trypan blue exclusion was used to determine cell viability over a 24 h period in Raji and HepG2 cells exposed to ammonium nitrate concentrations (25 μg/ml, 50 μg/ml, 100 μg/ml, 200 μg/ml, 400 μg/ml) and ultraviolet radiation intensities (250 J/m^2, 400 J/m^2, 600 J/m^2, 850 J/m^2), as described previously. Samples were taken from incubated culture flasks at time intervals of 1, 4, 8, 12, 18, and 24 h for viability counts.

2.4.2. Cell proliferation

Cell growth was observed over a 24 h period in Raji and HepG2 cells exposed to the previously described ammonium nitrate concentrations and ultraviolet intensities. Samples were taken from incubated culture flasks at time intervals of 1, 4, 8, 12, 18, and 24 h for cell counts.

2.5. Statistical analysis

Statistical analyses were performed with the SPSS for Windows statistical package, version 20.0 (SPSS, Inc., Chicago, IL). The analysis of variance test (ANOVA) and Student's *t*-test were performed to determine significant differences between the exposed and negative controls in each sample group. The level of statistical significance was set at $\alpha = 0.05$.

3. Results

3.1. Genotoxic effects of ammonium nitrate and ultraviolet radiation

There was a positive correlation between DNA damage and increasing concentrations of ammonium nitrate and ultraviolet radiation, as assessed by the comet assay in both Raji and HepG2 cell lines. In general, higher concentrations of ammonium nitrate or ultraviolet radiation induced higher levels of DNA damage, and lower levels of ammonium nitrate or ultraviolet radiation induced lower levels of DNA damage (Figs. 1, 3, 5 and 7; Tables 1 and 3). There were even greater levels of DNA damage observed when cells were exposed to both ammonium nitrate and ultraviolet radiation, with greater DNA damage when higher concentrations of one or both were used. We found a significant difference between all of the exposed samples and the negative controls in both Raji and HepG2 ammonium nitrate samples ($p < 0.001$).

3.2. Apoptosis at high concentrations of ammonium nitrate

At high concentrations (200 μg/ml, 400 μg/ml) of ammonium nitrate exposure in both Raji and HepG2 cell lines, DNA damage diminished with the addition of ultraviolet radiation (Figs. 1 and 3;