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Assessment of genetic toxicity with major inhalable mineral granules in A_{549} cells

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ABSTRACT

The toxicity of inhalable mineral granules has become a great interest topic of air pollution in the international. The majority of research in this area has focused on organic components that adhere to inhalable granules and on the pathogenicity of granules-associated heavy metal ions. However, as the main components of the mineral grains of dust, inhalable mineral granules is an important part of the dust, the granules themselves also represent important pathogenic factors. Whether the inhalable mineral granule in China has the genetic toxicity is unknown. To determine whether the mineral granules are genotoxic, a specific concentration range for 6 major components of the inhalable minerals in China were selected. The human lung adenocarcinoma cell line A_{549} was used to study granules genetic toxicity. Wright–Giemsa staining was used to evaluate cell morphology, the methyl thiazolyl tetrazolium (MTT) assay was used to determine any effects on cell proliferation, micronucleus tests were conducted to observe the effects of the minerals on the formation of chromosomal breaks and sister chromosome exchange (SCE) experiments were used to assess whether DNA synthesis was affected. The experimental results indicated the following: The membrane destruction, nuclear pyknosis, and mineral surface adhesion were observed in A_{549} cells treated with sericite or albite. In cells treated with quartz or montmorillonite, gaps between the cells were increased in size, the cells appeared more loosely arranged and became smaller than those of the control group, and a large amount of granules adsorbed to the cell surface. The inhibitory effect of the minerals tested on the proliferation of A_{549} cells ranked as follows, from most to least inhibitory: nano-montmorillonite, nano-SiO_2, nano-sericite, nano-quartz, albite, and nano-calcite. Mineral granules concentration correlated with an inhibition of the cell proliferation rate. As the concentration of the mineral granules increased, the incidence of micronuclei gradually increased for all minerals. All mineral granules tested caused DNA damage in the cells in a dose-dependent manner, as higher concentrations of mineral granules caused more DNA damage. These results indicate that inhalable mineral granules may be genotoxic to cells.

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1. Introduction

Air pollution has become one of the primary risk factors that affect public health, and increasing attention has been given to understanding the effects of air pollution exposure (Buschini et al., 2001; Gillissen et al., 2006; Lv et al., 2011). Inhalable granules have small granule size and large surface area. It can be entered the trachea and bronchi during normal respiration, then transported into the alveoli and the blood. These granules are closely associated with respiratory and cardiovascular diseases (Brown, 2009) and can cause germ cell genetic toxicity (De Boeck et al., 2000; Ben-Shlomo and Shanas, 2011) and induce cancer (Claxton and Woodall, 2007).

Epidemiological research has found that when the quality of the atmospheric environment decreases, especially when the particulate matter concentration in the air increases, the number of hospital admissions for respiratory causes rises sharply (Franck et al., 2011; Lee et al., 2014; Haghi et al., 2014). Exposure to these granules may adversely affect human health (Rittinghausen et al., 2013; Guidi et al., 2013).

Inhalable mineral granules is an important component of atmospheric particulate matter (Bergaya and Lagaly, 2013; Zhou and Keeling, 2013) especially there are serious sandstorms in northern China and scarcely research on it. The adsorption of proteins and nucleic acids on clay minerals and their interactions is important in biological applications for environment (Zhou, 2011; Yu et al., 2013). Some of the scholars have performed extensive research on the chemical compositions, sources and sinks of grains in China (Wu et al., 2014). It is mainly composed of various minerals; the main constituent of the grains is aluminosilicate granules, which account for 61.59% of the total granule count, whereas calcite and quartz granules account for approximately 13.59% of total granules

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(Chen et al., 2007). Long-term occupational level exposures to crystalline silicon dioxide can cause lung inflammation, edema, fibrosis and cancer (Jones and Bérubé, 2011). However, the biological genetic toxicity of inhalable mineral granules remains unknown.

Genetic toxicity refers to the physical and chemical factors in the environment acting on the organism, making the genetic material damaged in the chromosome, molecular and base level, and resulting in the changes of the genetic material. The genetic toxicity of granules involves toxic effects on chromosomes, DNA, genes and other levels of genetic material, including changes in chromosome structure, DNA damage and gene mutations (Wei and Meng, 2006; Gao et al., 2000; Moller et al., 2013; Bhattacharya et al., 2012). To evaluate the harm to cells by inhalable mineral granules which constitute the bulk of the granules, are important factors that should not be ignored. Scientifically objective evaluation of the components of inhalable mineral granules that pose a danger to cells could provide the basis for developing standards to detect and reduce it in the environment (Zhang et al., 2005; Sellamuthu et al., 2011; Maisanaba et al., 2014).

2. Experimental

2.1. Materials

2.1.1. Cell source

A_{549} cells are derived from a human small cell lung carcinoma and purchased from the Biomedical Laboratories of West China Medical Centre of Sichuan University.

2.1.2. Experimental mineral granules and preparation of mineral suspensions

Nano-quartz (KWC-Q) was collected from ErLangMiao mining area, Jiangyou, Sichuan, China. Nano-sericite (KWC-S) was purchased from Hai Yang powder technology co., LTD, Shenzhen, China. Calcite (KWC-C) was collected from PingTang buyi miao minority autonomous prefecture county, Guizhou, China. Nano-montmorillonite (KWC-M) was collected from Altai region, Xinjiang, China. Nano Silica (Nano-SiO_2) was given by Dr. Tongjiang Peng of the southwest university of science and technology.

Using X-ray diffraction to detect the mineral samples after it broken through 400 mesh, the results are shown in Fig. 1(a) and Fig. 1(a) by analysis the image of XRD, the mainly contents of mineral samples are shown in Table. 1.

The source material was ground for 10 h using a clean horizontal ball mill. A Malvern laser granule size analyser was then used to measure granularity, after which the material was suction-filtered and dried. The dried sample was then ground a second time and passed through a 200-mesh screen and packaged for later use. Before using the minerals, it dried in an oven (T = 110 °C) and then irradiated under a UV light for 48 h.

Serum-free medium was used to prepare the mineral granules for use. Briefly, the medium-mineral mixtures were swirled for 30 min to produce a suspension. At the end of this process, no aggregation of the granules was observed, and the average granule diameter was less than 10 μm.

2.1.3. Reagents and instruments

Improved RPMI1640 culture medium containing foetal bovine serum was supplied by HyClone, America. Methyl thiazolyl tetrazolium (MTT), penicillin–streptomycin solution and 0.25% trypsin were supplied by Beyotime Institute of Biotechnology, China.

An automated enzyme immunoassay instrument was purchased from Thermo Multiskan, America. An ultra-low temperature high-speed centrifuge and a CO_2 incubator were purchased from Thermo Electron Corporation, America. An inverted microscope was purchased from Chongqing Optical Instrument Factory, China. A horizontal planet ball mill was purchased from Chunlong Instruments, China. A Malvin laser granule size MS2000 analyser was purchased from Malvin Instruments, UK. A fluorescence microscope was purchased from Olympus, Japan. An automated enzyme immunoassay instrument was purchased from Bio–Rad, USA. A fluorescence microscope was purchased from Zesis, Germany.

2.2 Methods

2.2.1 Wright–Giemsa staining

Cells in the logarithmic growth phase were suspended at a concentration of 1.0×10^5 cells mL^{-1} and seeded into culture dishes containing cover slips. The cultures were incubated for 48 h to allow the cells to adhere to the cover slip. The disinfected mineral granule suspensions were added to the culture dish at final concentrations of 200 μg mL^{-1}. Each experiment included a negative control group. After an additional of 48 h incubation, the cover slips were removed, gently washed in PBS, dried, fixed and stained with Wright–Giemsa stain for 10 min. The cover slips were then washed again by PBS (pH 7.2), air-dried. And then the incubated cells on cover slips were observed by light microscopy.

2.2.2 MTT test

Cells in the logarithmic growth phase were suspended at a concentration of 1.0×10^5 cells mL^{-1}, seeded in 96-well plates and then incubated at 37 °C in 5% CO_2 for 48 h. After the cells adhered to the plates, the medium was aspirated and discarded, and then the serum-free mineral granule suspensions were added to the cells at final concentrations ranging from 50 to 400 μg mL^{-1}. After an additional incubation for 48 h, 20 μL MTT solution was added to each well of the culture plate. The cultures were incubated for 2 h, after which the culture medium from each well was replaced with 200 μL dimethyl sulfoxide. The plates were mixed by shaking, and the absorbance of each well of the culture plate was measured using an automated enzyme immunoassay instrument at a wavelength of 490 nm. The relative survival rates of the cells were calculated according to the following formula:

$$\text{Cell viability } \% = (A_0 - A_1)/A_0 \times 100\%,$$

where A_0 was the absorbance of control group, A_1 the absorbance of mineral treatment group.

2.2.3 Micronucleus test

Cells in the logarithmic growth phase were suspended at a concentration of 1.0×10^5 cells mL^{-1}, seeded in culture dishes and incubated at 37 °C in 5% CO_2 for 48 h. After the cells adhered, the culture medium was aspirated and discarded, and then the 6 different serum-free mineral granule suspensions were added at a final concentration of 200 μg mL^{-1}. Cell culture medium was added to the negative control group, and 200 μg mL^{-1} cyclophosphamide (CP) was added to the positive control group. The cultures were incubated for an additional 48 h, washed with PBS and then air-dried. The cells were fixed with methanol/formaldehyde (vol ratio, 3:1) for up to 30 min and then stained with Wright–Giemsa stain. The A_{549} cell morphology was examined by light microscopy. In addition, 3000 cells from each slide were examined, and the numbers of cells containing micronuclei were counted. The results were expressed as a percentage of the total cells according to the following formula:

$$FMN = n_1/n_0 \times 100\%,$$

where n_1 was the number of cells containing micronuclei, n_0 the total number of cells observed.

2.2.4 SCGE test

A_{549} cells were exposed to suspensions of 200 μg mL^{-1} mineral granules for 48 h and then harvested with 0.25% trypsin. The cells were transferred to a centrifuge tube and centrifuged at 1000 r min^{-1} for 10 min. The supernatant was discarded, and the pellet was

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