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Cellular responses of human astrocytoma cells to dust from the Acheson process: An *in vitro* study

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ABSTRACT

Silicon carbide (SiC) is largely used in various products such as diesel particulate filters and solar panels. It is produced through the Acheson process where aerosolized fractions of SiC and other by-products are generated in the work environment and may potentially affect the workers' health. In this study, dust was collected directly on a filter in a furnace hall over a time period of 24 h. The collected dust was characterized by scanning electron microscopy and found to contain a high content of graphite particles, and carbon and silicon containing particles. Only 6% was classified as SiC, whereof only 10% had a fibrous structure. To study effects of exposure beyond the respiratory system, neurotoxic effects on human astrocytic cells, were investigated. Both low, occupationally relevant, and high doses from 9E-6 μg/cm^2 up to 4.5 μg/cm^2 were used, respectively. Cytotoxicity assay indicated no effects of low doses but an effect of the higher doses after 24 h. Furthermore, investigation of intracellular reactive oxygen species (ROS) indicated no effects with low doses, whereas a higher dose of 0.9 μg/cm^2 induced a significant increase in ROS and DNA damage. In summary, low doses of dust from the Acheson process may exert no or little toxic effects, at least experimentally in the laboratory on human astrocytes. However, higher doses have implications and are likely a result of the complex composition of the dust.

1. Introduction

Silicon carbide (SiC) produced by the Acheson process is a well-known ceramic material known for its properties such as chemical inertness, elevated thermal stability and excellent mechanical properties. It is used in a wide variety of industrial purposes both in the ceramic and composite material fields (Oliveros et al., 2013). Furthermore, there is an increased interest in the use of micro and nanoscale SiC materials in the areas of ceramics, electronics and catalysis. Thus, as SiC likely will continue to be used in a various number of products, research on potential health effects due to occupational exposure will be of importance.

Most toxicological effects related to SiC from the Acheson process have focused on the respiratory effects and most of the knowledge is on SiC whiskers. Analyses on the effects of dust exposure of workers in the SiC industry have indicated increased loss of lung function, increased mortality from non-malignant respiratory diseases and increased incidence of lung cancer (Bugge et al., 2011, 2012; Johnsen et al., 2013). Due to their ability to cause lung cancer, occupational exposure associated with the Acheson process has been classified as carcinogenic to humans by the International Agency for Research on Cancer (Grosse et al., 2014).

Subchronic inhalation and intrapleural injection of SiC whiskers in rats induced inflammatory lesions, thickening of the pleural wall, pleural fibrosis and mesotheliomas (Lapin et al., 1991; Johnson and Hahn, 1996). Studies on SiC microparticles showed that the particles triggered lung inflammation (Cullen et al., 1997), granulomas (Vaughan et al., 1993), fibrotic changes in the lungs (Akiyama et al., 2007), exerted cytotoxic and genotoxic effects (Vaughan et al., 1993), induced reactive oxygen species (ROS) (Svensson et al., 1997) and increased the expression of inflammatory cytokines (Cullen et al., 1997). Furthermore, accumulation of nanoscale SiC particles was observed in lung epithelial cells and induced ROS and DNA damage (Fan et al., 2008; Barillet et al., 2010).

There is little knowledge on potential neurotoxic effects of dust from the Acheson process. Dust emitted from the Acheson process has several components, including silica particles that may have an

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impact on the central nervous system. Nanoscale silica particles showed increased oxidative damage and inflammatory responses in the brain after intranasal instillation in experimental animals (Wu et al., 2011). Furthermore, uptake of silica nanoparticles decreased neuron cell viability, induced ROS, apoptosis, and increased Alzheimer-like pathology (Yang et al., 2014). Supporting this, a recent study showed that silica nanoparticles can cross the blood-brain barrier and induce neuronal cell damage (Zhou et al., 2016). Other elements, such as carbon, aluminum and vanadium, that can be part of occupational exposure in the Acheson furnace hall have all been shown to have neurotoxic effects (Campbell, 2002; Garcia et al., 2005; Onoda et al., 2017).

We sought to investigate the effects of dust collected in the furnace hall of a SiC facility on a human astrocytic cell line cultured in the laboratory. The human cells were exposed to a range of doses, including low occupationally relevant, and high doses. The results showed minimal dose-dependent toxicity, ROS production and DNA damage.

2. Materials and methods

2.1. Collection of the Acheson dust

A 142 mm stainless steel filter holder, YY3014236 (Merck Millipore, Massachusetts, USA) was placed in the middle of an Acheson furnace hall close to one of the furnaces. A 142 mm diameter polycarbonate filter with pore size 10 μm was placed on top of the filter holder and then connected to a high output vacuum pump, WP6222050 (Merck Milllipore, Massachusetts, USA), with adjustable output. This was left on for a period of 24 h where dust was collected from the filter three times during this period. The dust can be considered as an impure powder with no industrial application and is representative of the airborne dust that can be inhaled in the work environment of a furnace hall.

2.2. Preparation of the Acheson dust for characterization and cell culture experiments

The dust was weighed and for dispersion a slightly modified version of the NANOGENOTOX protocol was used (Jensen et al., 2011; Phuyal et al., 2017). Briefly, to obtain well-dispersed particles a solution of sterile-filtered 0.05% Bovine Serum Albumin (BSA) (diluted in H_2O, m/v) was added to obtain a stock solution of 1 mg/ml. After a brief vortexing, the solution was sonicated using a probe sonicator at 10% amplitude (Sonifier 450S, Branson Ultrasonics, Danbury, USA) for 15 min. For each single experiment a freshly prepared stock was used. In the cell culture experiments, controls were exposed to the highest volume of 0.05% BSA that was used to prepare the highest dose of Acheson dust for exposure. For the highest dose of Acheson dust (4.5 μg/cm^2) this corresponded to a final BSA concentration of 0.000675% in the cell culture media.

2.3. Acheson dust characterization

2.3.1. SEM

The Acheson dust was prepared as follows: a volume corresponding to 100 μg was taken from a 1 mg/ml stock dispersed in 0.05% BSA which was sonicated as described above followed by filtering on a 47 mm Whatman Nuclepore polycarbonate filter with 50 nm pore size. Thereafter the filter was coated with a thin platinum film in a sputter coater (Cressington 208HR sputter coater, UK). Specimens of 10 × 10 mm were cut from the filter and gently fixed on aluminum specimen stubs with double-sided carbon adhesive discs. The specimens were analyzed with a Hitachi SU 6600 (Ibaraki-ken, Japan) field emission scanning electron microscope (FE-SEM) equipped with a Bruker energy-dispersive X-ray detector. The instrument was operated under the following conditions: accelerating voltage 15 keV and working distance 10 mm. High resolution images of the particles were obtained by acquiring at slow scanning speed. Initially, specimens were examined in the SEM to determine their morphology and size. The chemical composition of the Acheson dust particles was obtained by energy dispersive x-ray spectroscopy (EDX).

2.3.2. Dynamic light scattering

To obtain information on the dusts' hydrodynamic size distribution after dispersion, ZetaSizer Nano ZS (Malvern Instruments Ltd, UK) was used. Immediately after sonication 1 ml of the sonicated solution was pipetted into a cuvette, left on the bench for 5 min and was thereafter left for 5 min in the ZetaSizer apparatus before measuring over 10 cycles. ZetaSizer software (Malvern Instruments Ltd, UK) was used to analyze the data. The results shown are from three independent measurements.

2.4. Cells and cell culture

The human astrocytoma 1321N1 cell line was purchased from Sigma-Aldrich (catalogue no. 86030402). These are glial cells from a human brain astrocytoma that was initially isolated in 1972 as a sub clone of the cell line 1181N1 (Macintyre et al., 1972). Cells were routinely kept in a humidified 5% CO2 and 95% air incubator at 37 °C in Dulbecco's Modified Eagle's Medium (DMEM, Fisher Scientific) containing 10% fetal bovine serum (FBS, Biochrom), 50 U/ml penicillin and 50 μg/ml streptomycin (Thermo Scientific). The passage number of the cells was kept below 30.

2.5. Estimation of dust doses used for cell culture experiments

The doses used for cell culture exposures were kept low to mimic occupational exposure and were calculated following a mathematical calculation modified from Antonini and coworkers (Antonini et al., 2010, 2013) to determine the daily lung burden of a worker working 8 h per day. Incorporated factors were the occupational exposure limit for respirable dust in the silicon carbide industry (0.5 mg/m^3), human minute ventilation volume (20.000 ml/min × E-6 m^3/ml), the exposure duration (8 h/day), the deposition efficiency (set to 20%; (Oberdorster et al., 2005a, 2005b)).

The daily deposited dose was:

$$0.5\ \text{mg/m}^3 \times (20.000\ \text{ml/min} \times 10^{-6}\ \text{m}^3\text{/ml}) \times (8\ \text{h} \times 60\ \text{min/h}) \times 0.20 = 0.96\ \text{mg}$$

When using the surface area of the alveolar epithelium (human 102 m^2 (Stone et al., 1992)) this leads to a deposited dose of 9E-4 μg/cm^2 (0.96 mg/1.020.000 cm^2 = 0.9E-6 mg/cm^2 → 0.0009 μg/cm^2). This dose was set as 1 x and the other doses (0×, 0.01×, 0.1×, 10×, 100×, 1000×, 5000×) used in this study were calculated accordingly. Thus cells were exposed to 0, 9E-6, 9E-5, 9E-4, 9E-3, 0.09, 0.9 and 4.5 μg/cm^2 taking into account the surface area of the cell culture dish, respectively.

2.6. Cytotoxicity assay

For each toxicity experiment 5000 cells/well were seeded in triplicate in black 96-well plates with a transparent bottom (Nunclon, Thermo Scientific). Cells were allowed to attach for 24 h prior to addition of dispersed Acheson dust at the indicated doses.

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