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Factors of concern in a human 3D cellular airway model exposed to aerosols of nanoparticles

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ABSTRACT

Mucilair 3D bronchial airway models, cultured at an air-liquid interface, were exposed to aerosols of copper oxide (CuO) nanoparticles in Vitrocell air exposure modules. Four cell donors, four exposure modules and four exposure concentrations were varied within four different exposure sessions using a statistical experimental design called a hyper-Graeco-Latin square. Analysis of variance techniques were used to investigate the effects of these factors on release and RNA expression of inflammation markers monocyte chemoattractant protein-1 (MCP-1) interleukines 6 and 8 (IL-6 and IL-8) an cytotoxicity marker lactate dehydrogenase (LDH) determined 24 h after exposure. The same techniques were also used to conduct a global analysis on RNA expressions of 10,000 genes.

There were no major signs of cytotoxicity. Release of IL-6 and MCP-1 was affected by CuO concentration, and, for MCP-1, by donor variation. IL-8 release was not affected by these factors. However, gene expression of all three inflammation markers was strongly affected by CuO concentration but not by the other factors. Further, among the 10,000 genes involved in the global analysis of RNA expression, 1736 were affected by CuO concentration, 704 by donor variation and 269 by variation among exposure sessions.

The statistical design permitted the assessment of the effect of CuO nanoparticles on 3D airway models independently of technical or experimental sources of variation. We recommend using such a design to address all potential sources of variation. This is especially recommended if test materials are expected to be less toxic than CuO, because the variation among the concentration levels could then be close to the variation among donors or exposure sessions.

1. Introduction

Human 3D airway models are fully differentiated and functional models of the respiratory epithelium. The functionality includes metabolic activity, mucus production and cilia beating (Kuper et al., 2015; Reus et al., 2014). The models are cultured at an air-liquid interface so that they allow relevant exposure via air. It is anticipated that they realistically predict bioavailability of inhaled compounds. To substantiate any claim to this extent, however, requires demonstration of a clear dose-response relationship for adverse effects such as inflammation, based on several concentrations of the compound under investigation. This introduces several methodological problems that need to be addressed. First, the 3D models originate from individual donors. Donor variation is a well-known source of variation in in vitro experiments. Therefore, we need to assess unequivocally which part of the variation in the response is due to the substance and its concentrations and which part is due to the variation introduced by the donors.

A second problem that needs to be addressed is the variation introduced by the equipment and the experimental procedure. It is inevitable that several exposure modules for the cell material are needed to conduct a fully-fledged exposure study. At the same time, not all the exposures can be conducted simultaneously, so that several exposure sessions are needed to complete the study. What is needed is a clear assessment of the amount of variation introduced by exposure module and exposure session. In case these technical sources of variation appear substantial, future experiments need to be set up such that these sources of variation do not affect the comparison between the concentration levels of the compound.

A statistical design of experiments called a hyper-Graeco-Latin square permits the simultaneous assessment of five potential sources of variation in a modest amount of experimental effort. The purpose of this paper is to show how an experimental study can be conducted that

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simultaneously assesses the contributions of five such sources of variation to the responses under study, and to show how the data from such a study can be analysed so that these contributions can be quantified. The sources of variation include compound concentration, donor, exposure session, exposure module and a factor that captures random variation. As a vehicle for demonstration, we use exposure of the MucilAir 3D bronchial model to copper oxide nanoparticles. The main responses were both the release and the gene expression of the cytokines interleukine 6 (IL-6), interleukine 8 (IL-8) and monocyte chemoattractant protein-1 (MCP-1) as markers of inflammation. We checked cytotoxicity by studying release of lactate dehydrogenase (LDH) and conducted a global assessment of RNA expression in 10,000 genes. The total number of exposures in the statistical design was 16, while the full study, taking incubator controls and an extra session to determine deposition rates into account, included 24 exposures. Yet, the study permitted the demonstration of clear dose-response effects of nano-CuO particles along with the assessment of donor differences, differences among exposure sessions and differences among exposure modules.

2. Methods

2.1. Experimental equipment

A schematic of the experimental equipment is shown in Fig. 1. The equipment consists of a generator for the aerosol, an air-liquid interface exposure system and the MucilAir 3D human bronchial epithelial model. A comprehensive description of the equipment has been published elsewhere (Kooter et al., 2016). Details specific to the present application follow below.

2.1.1. Aerosol generation

The test atmosphere was generated by aerosolizing nano-CuO, 33 nm primary particle size (batch MKBH9047V and MKBJ4678V from one production of the external supplier, Sigma Aldrich) using a turntable dust feeder (Reist and Taylor, 2000) and an eductor (Fox Valve Development Corp., Dover, NJ, USA (Cheng et al., 1989)) supplied with humidified compressed air at 0.5–0.6 kg/cm^2. The total air flow was 60.1–65.8 l/min for the generation of the nano-CuO test atmosphere (day 1, session 1 and 2 and day 2, session 1: pressure: 0.6 kg/cm^2, resulting airflow: 65.8 l/min. Day 2, session 2 and 3: 0.5 kg/cm^2, resulting airflow 60.1 l/min). The test atmosphere was introduced at the top of the buffer chamber, directed downward.

Fig. 1 shows that an air control, a high-concentration, a mid-concentration and a low concentration flow were realized simultaneously. The test atmosphere for the high concentration flow was extracted from the buffer chamber using a mass flow controller (Bronkhorst Hi Tec, Ruurlo, The Netherlands) connected to a vacuum source.

For the mid- and low concentration flows, the test atmosphere was diluted using an AirVac eductor (Air-Vac Engineering Company, Seymour, CT USA). Adjustments were made to ensure 50% relative humidity among all flows. Measurements of temperature and relative humidity were made with a Testo RH/T device (Testo 635, TESTO GmbH & Co, Lenzkirch, Schwarzwald, Germany).

The actual concentration of the test material in the buffer chamber and for high, mid and low concentration flows was measured by gravimetric analysis using fibre glass filters (Sartorius, 13400-47). In addition, measurements of particle size in the buffer chamber were performed using a scanning mobility particle sizer (SMPS model 3080, measuring range 14–673 nm, TSI Inc., Shoreview MN, USA) and an aerodynamic particle sizer (APS model 3321, measuring range 0.5–15.4 µm, TSI Inc., Shoreview MN, USA).

For electron microscopic analysis, samples were collected using IOM inhalable samplers (SKC Inc., USA) on nickel coated track-etched polycarbonate filters (0.1 µm 25 mm, Nuclepore) with a flow rate of 100 ml/min. Filters were analysed with scanning electron microscopy as reported before (Peters et al., 2014).

2.1.2. Air-liquid interface exposure system

To expose cells at an air-liquid interface to copper oxide aerosols, four Vitrocell® modules (Vitrocell Systems GmbH, Waldkirch, Germany) were used, hereafter referred to as test blocks. Each test block supports three inserts for exposure with adaptable well size; see the rectangles with three circles each in Fig. 1. The colored schematic below the rectangles show the two parts of each test block: a lower chamber, in which three glass medium-containing wells are surrounded by circulating 37 °C water, and an upper chamber, through which the aerosol or medical air, which is a colorless, odorless, and tasteless gas similar in composition to the air that we breathe, is drawn into the three individual exposure inserts at a flow rate of 1.5 ml/min per insert (MucilAir, 24 well size).

To determine the deposited dose, a separate experiment (day 2, exposure session 3) was performed with MucilAir inserts under the same exposure conditions as applied in the main experiment. In this experiment, the total concentration of Cu was determined with an Element XR High Resolution Inductively Coupled Plasma Mass Spectrometer (Thermo, Bremen, Germany) in the basal medium collected during the exposure, the basal medium collected during the post-exposure and the cell lysate, respectively.

2.1.3. MucilAir™ 3D human bronchial epithelial model

MucilAir™ fully differentiated bronchial epithelial models (Epithelix Sárl, Geneva, Switzerland), reconstituted from primary human cells from healthy donors, were used for air-liquid exposure to CuO. Cells

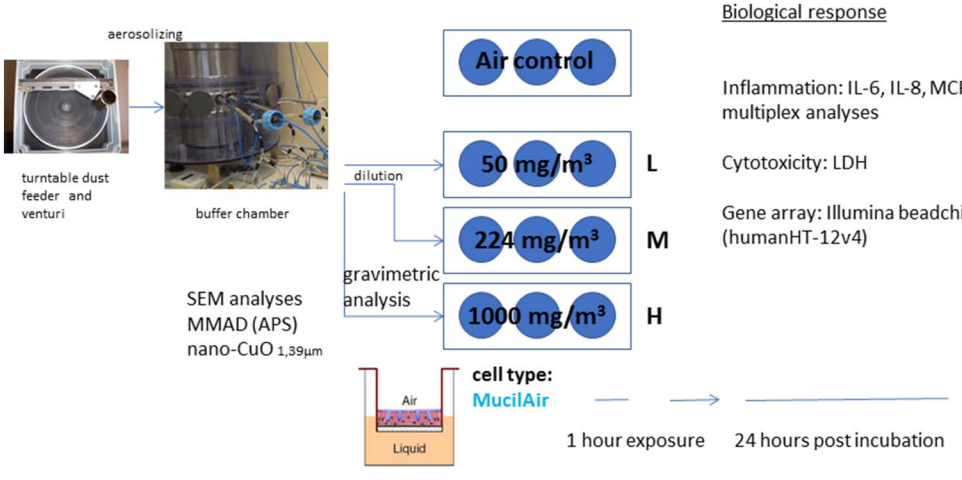

Fig. 1. Schematic drawing of the experimental setup used for CuO exposure (adapted from (Kooter et al., 2016)). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)