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Pulmonary responses to printer toner particles in mice after intratracheal instillation

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ABSTRACT

The release of ultrafine particles from office equipment is currently receiving great concerns due to its potential threat to human health when inhaled. Printer toner is one of the largest consumables in daily office work, and the particles released from printers and photocopiers may pose damage to respiratory system. In this study, we found the particles can be released into the surrounding environment during the printing process and the concentrations of $PM_{2.5}$ and PM_{10} particles increased obviously. To evaluate the time-course pulmonary responses caused by toner particles, the toner suspension was instilled into the lungs of the male mice through intratracheally instillation every other day for four times and the pulmonary responses of the lung were monitored at days 9, 28, 56 and 84. Indeed, mice treated with toner particles displayed a slower body weight growth rate during the recovery phase. The total cell number in bronchoalveolar lavage fluids (BALF) of toner-exposed groups was much higher than the saline-treated groups. The total protein, lactate dehydrogenase and acid phosphatase in BALF exhibited significant changes ($p < 0.05$ or $p < 0.01$) at different time points. The nitric oxide synthase, interleukin 1-beta, and interleukin 6 in the lung tissue of the toner-exposed groups also exhibited significant changes ($p < 0.05$ or $p < 0.01$). The pathological examination showed that toner particles can adhere to the alveolar septal walls, then enter into the alveoli and cause pulmonary lesion. During the experimental period, particles phagocytosed by alveolar macrophages (AMs) led to an increase of both AMs number and apoptosis. The pulmonary stress still remained over time even with a clearance period for 12 weeks. These results indicate that exposure to toner particles can inhibit the normal growth of the mice and induce significant inflammatory responses and lesion in the lung tissues. The health and safety effects from working indoors in offices with fumes and particles released from photocopiers and printers need to be paid more attention.

1. Introduction

With the rapid development of information technology (IT), the affiliated output equipment, which is mainly composed of laser printers, inkjet printers, multifunctional photocopiers and so on, has become the third largest IT market. Various types of printers are widely used in offices and homes around the world and have become standard indoor electronic equipment. They not only bring convenience to humans, but also have been suspected as a potential source of indoor air pollutants (Wolkoff et al., 1992; Wolkoff, 1999). Some case reports and a few studies have suggested that some common office environment exposures, such as exposure to carbonless copy (CCP) (Morgan and Camp, 1986; Shehade et al., 1987; LaMarte et al., 1988; Skov et al., 1989; Kanerva et al., 1993; Jaakkola and Jaakkola, 1999) and fumes from photocopies and printers (FPP) affect health adversely (Skov et al., 1989; Jaakkola and Jaakkola, 1999; Yassi and Warrington, 1988; Fisk et al., 2004; Stenerg et al., 1993). In fact, during the printing process, the printers not only produce FPP, but also emit a variety of particles (Brown, 1999; Kagi et al., 2007; Lee et al., 2001; Eggert et al., 1990; Wensing et al., 2006; Uhde et al., 2006), which partially come from toner. As we know, toner consists of very small particles of thermoplastic polymer, usually styrene–acrylate copolymer that are fixed on the paper by fusing. Black toner contains black carbon or iron oxide as pigments. In addition to these main constituents, toner contains

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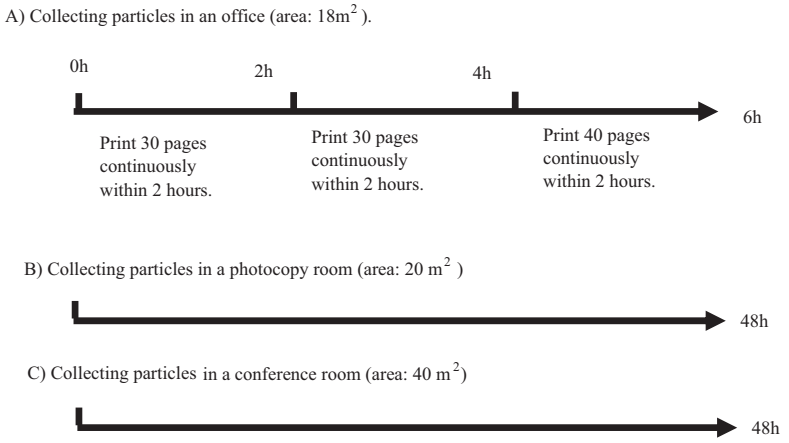

Fig. 1. Detail experimental scheme of the collecting of particles during the printing process by two printers. (A) Collecting particles in an office (area: 18 m^2). (B) Collecting particles in a photocopying room (area: 20 m^2). (C) Collecting particles in a conference room (area: 40 m^2).

various additives such as wax and silica, but also small amounts of specific metal salts to control the electromagnetic properties. Typically, about 75% of the toner is transferred to the photoconductive drum. However, toner particles that do not adhere to the drum become available for emission in the indoor air. This may lead to users being exposed to different concentrations of emitted particles. Kagi et al. (2007) confirmed that an increase in the concentration of ultrafine particle number in the printing process of the printers. Especially for the case of around 50 nm particles, particulate concentration increased greatly during printing. Lee and Hsu (2007) found that the emitted particles were much smaller than the original toner powders, which was similar to the above data (Kagi et al., 2007). He et al. (2007) investigated the particle emission characteristics of office printers and found the particle emission rates are printer-type specific and are affected by toner coverage and cartridge age.

The respiratory system is sensitive to bacteria, viruses, and many airborne particles that can be inhaled. Worldwide epidemiological studies show a consistent increase in cardiac and respiratory morbidity and mortality from exposure to particulate matter (PM) (Dockery et al., 1993; Samet et al., 2000; Brook et al., 2004). PM air pollutants have been shown to exacerbate a variety of pulmonary disorders, including chronic obstructive pulmonary disease (Schwartz, 1994; Sunyer and Basagaňa, 2001), asthma (Lipsett et al., 1997; Peters et al., 1997), and lower respiratory tract infections. We have learnt that the printers can emit a lot of ultrafine particles during the printing process. The unusual physicochemical properties of nanoparticles (such as larger surface area, surface reactivity and so on), which are differ substantially from their bulk materials of the same composition, allow them to interact with biological systems and the environment, with the potential to generate toxicity (Nel et al., 2006). People spend approximately 80% of their time in indoor environment where the levels of air pollutants can be several hundred times higher than outdoor (U.S. Environmental Protection Agency, 1987, 1995). Toner, as one of the largest consumables in daily office work, its demand is increasing with the popularity of printers and photocopiers. It is estimated that the global demand for toner is around 240,000–260,000 tons. So its release and influence on the respiratory system cannot be ignored.

Therefore, the aim of this study was to evaluate the pulmonary responses of toner particles via an animal model by intratracheal instillation. We firstly monitored the concentrations of particles emitted from printing process using $PM_{2.5}$ and PM_{10} particle samplers. Then the toner particles suspension in physiological saline solution was administered into the lungs of mice by non-surgical intratracheal instillation. The mice growth, lung inflammatory and fibrotic responses, expression of pro-inflammatory cytokines and pathological changes were analyzed to evaluate the time-course pulmonary responses caused by toner particles.

2. Materials and methods

2.1. Toner particles

The toner was purchased from Beijing Laisheng High-tech Co., Ltd. The average size and composition of the toner particles were determined by transmission electron microscopy (TEM, Tecnai G220S-TWIN) and energy dispersive X-ray (EDX) analysis at an electron beam voltage of 200 kV. The particles were also analyzed by environmental scanning electron microscope (ESEM, Quanta 200 FEG) at low vacuum condition to observe the surface morphology. The metal impurity of the particles was determined by inductively coupled plasma–mass spectrometry (ICP–MS, Thermo Elemental X7). The crystal structure was determined on a X-ray diffraction (XRD, Rigaku D-MAX 2500) with CuKα radiation ($\lambda = 0.154$ nm) using a generator voltage of 45 kV and a generator current of 250 mA.

2.2. Collecting particles during the printing process

$PM_{2.5}$ and PM_{10} particle samplers (Dick Company of Beijing Geological Instrument Factory, PM_{10}-2 model and $PM_{2.5}$-2 model) were used to collect particles emitted from the printing process. Membranes used in the experiment were produced by Pall Life Sciences (TISSUQUARTA 2500QAT-UP).

The first case was to collect particles in the office (area: 18 m^2). Two printers were put on the desk closely. The sampling heads of the two samplers were positioned 0.5 m above the printers. The two printers printed every 2 h. For the first and the second block of 2 h, each printer printed 30 pages. In the last block of 2 h, each printer printed 40 pages. The collecting process lasted 6 h and repeated 3 times. The second case concerns the particles collection in the photocopy room (area: 20 m^2) for 48 h. In this period, totally 595 pages were copied. The third case was to collect particles in the conference room (area: 40 m^2) for 48 h. During the whole collection experiment, nobody can be allowed to enter the rooms. The detailed experimental scheme is shown in Fig. 1.

Membranes were put into a desiccator at least 24 h before use. They were weighed by a dedicated scale (Mettler Toledo XS-105) with the resolution of 0.001 mg. Each membrane was weighed 3 times. After sampling, membranes were put into a desiccator for 24 h, and weighed again. This process was repeated 3 times.

2.3. Animal experiment

2.3.1. Animals

Male ICR mice (body weights of 20–22 g) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. The animals were fed in standard cages in an air-conditioned room (20 ± 2 °C, 50–70% relative humidity, with a 12-h light/dark cycle). Commercial sterilized food for mice and deionized water were available *ad libitum*. All procedures used in this experiment were compliant with the local ethics committee. Animals were acclimated to this environment for one week prior to treatment.

Mice were randomly divided into non-exposure and exposure groups. Non-exposure group corresponded to a normal control group containing 8 animals and a vehicle control group of physiological saline solution and exposure groups included toner suspension exposure group. Each exposure group had 24 animals. Toner particles were suspended in physiological saline solution. The toner suspension was