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Research paper

Chronic upper airway inflammation and systemic oxidative stress from nanoparticles in photocopier operators: Mechanistic insights

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ABSTRACT

Background: Several recent studies have linked emissions from printing equipment with upper airway inflammation and systemic oxidative stress in healthy humans, lung inflammation in mice, and cytotoxicity, induction of inflammatory markers and epigenetic changes in human cell lines. Acute exposures have lead to upper airway inflammation and systemic oxidative stress, which for certain markers took longer than 24–36 h post-exposure to clear.

Objective: In this follow-up work, we determined: i) whether chronic exposures to nanoparticles from copiers lead to chronic upper airway inflammation and systemic oxidative stress; and ii) whether expression patterns of biomarkers for such stresses change during transition from acute to chronic exposures.

Methods: Six permanent employees from three copy centers and eleven controls participated in the study. Nasal lavage and urine samples were collected on Monday morning (pre-shift, Mo-AM) and evening (post-shift, Mo-PM), as well as at the end of the workweek (Fr-PM), over three random weeks. The matched controls were sampled over one week. Nasal lavage samples were analyzed for a panel of 14 pro-inflammatory cytokines/chemokines, inflammatory cells, and total protein. Urine samples were analyzed for *8-OH-dG*, a biomarker of systemic oxidative stress. Detailed quantitative exposure assessment to airborne nanoparticles was conducted for a whole week, and included size distribution, size-fractionated aerosol collection, extensive chemical analysis, and lung burden estimates.

Results: The daily geometric mean total particle number concentration varied between 14,600–21,860 particles/cm^3, 1.7–12.1 times greater than background, with maxima up to 143,000 particles/cm^3. Mass concentration of the nanoscale fraction was in the 1–10 μg/m^3 range. Chemical composition of the nanoparticle fraction was comprised mostly of organic compounds, mixed with several engineered nanoparticles, which contributed a metal content ranging from 2 to 8% of the total particulate mass.

Five out of the 14 inflammatory cytokines, namely IL-6, IL-8, TNFα, IL-1β and Eotaxin, were significantly elevated in the nasal lavage samples of the chronically exposed copier operators ($p < 0.0001$) relative to controls. One cytokine, G-CSF, was significantly down regulated ($p < 0.0001$) in copier operators ($p < 0.05$). The level of all six cytokines did not change significantly across days (i.e. Mo-AM vs. Mo-PM, and Mo-AM vs. Fr-PM) and across weeks in chronically exposed individuals. In addition, there were significant ($p < 0.0001$) increases in inflammatory cell infiltration (2.7 fold) in nasal lavage samples and *8-OH-dG* in (4.3 fold) in urine samples.

Conclusion: Chronic upper airway inflammation and systemic oxidative stress were documented in photocopier operators chronically exposed to nanoparticles. These findings agree with the recent toxicological literature on printer-emitted particles and medical case reports, and call for an industry-wide study of the health effects resulting from exposure to printer-emitted particles in chronically exposed workers. Inflammatory markers point to possible involvement of toll-like receptors, particularly TLR-4, oxidative stress, and the Nf-kB pathway in mediating airway tissue inflammation.

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1. Introduction

Toner-based printing devices, namely laser printers and photocopiers, emit high levels of nanoparticles (Bello et al., 2013; Martin et al., 2015; Pirela et al., 2014). Printing and photocopying, like many other industries, have shifted fully to nano-enabled toner formulations that incorporate several engineered nanoparticles in toner and paper (Martin et al., 2015; Pirela et al., 2014). Exposures of photocopier operators are primarily to nanoparticles. Volatile organic compounds (VOCs and ozone), historically major contaminants emitted from photocopiers, have been reduced over the years as a result of toner reformulations and other technological modifications (Martin et al., 2016). Nanoparticles are at present the main contaminant emitted from photocopiers.

The airborne nanoscale fraction of emissions from copying equipment is a complex mixture. It contains incidental nanoparticles generated as a result of the condensation of semi-volatile organic compounds originating from thermal decomposition of the polymeric material of the toner, as well as engineered nanoparticles added to the toners (Martin et al., 2015). Several engineered nanoparticles have been identified in toners, including among others iron oxide (Fe_2O_3), titanium dioxide (TiO_2), silicon dioxide (fumed silica or SiO_2), aluminum oxide (alumina or Al_2O_3), cerium oxide (ceria or CeO_2), and oxides of copper (Cu), manganese (Mn), and nickel (Ni). Chronic health effects of such nanoparticle exposures in full-time copier operators and frequent printer users have not been studied and, mechanistically, remain poorly understood, in part because responses associated with repeated exposures have received attention only in the past few years. In this paper, we will refer to photocopiers and printers collectively as toner-based hard copy devices. Indeed, we have shown in two separate studies (Martin et al., 2015; Pirela et al., 2014) that emissions from such devices are chemically and morphologically quite similar, and the only major difference between the two technologies is the volume of printing or photocopying, i.e. the resulting exposure profiles.

In an earlier paper, we demonstrated that acute exposures to nanoparticles from photocopying leads to acute upper airway inflammation and systemic oxidative stress in young, healthy volunteers (Khatri et al., 2013a). In that study, a single exposure episode of up to six hours with a daily average exposure of 20,000 to 30,000 particles/cm^3 triggered a notable increase 6-h post-exposure of several inflammatory markers in the nasal lavage, including interlukin-6 (IL-6), interlukin-8 (IL-8), tumor necrosis factor α (TNFα), interlukin-1β (IL-1β), granulocyte-colony stimulating factor (G-CSF), epidermal growth factor (EGF), interlukin-10 (IL-10), monocyte chemoattractant protein-1 (MCP1), fractalkine and vascular endothelial growth factor (VEGF). Most of these cytokines - TNFα, IL-1β, G-CSF, IL-10, MCP1 and VEGF - reached baseline levels within 24–36 h, whereas four cytokines - IL-6, IL-8, EGF, and fractalkine - remained elevated even 30 h post-exposure. Urinary 8-hydroxy-2′-deoxyguanosine (*8-OH dG*), a marker of systemic oxidative stress, as well as neutrophil infiltration and total protein in the nasal lavage also increased 6 h post-exposure and returned to pre-exposure level by 30 h post-exposure. These responses exhibited similar profiles to those of cytokines. Based on such acute responses, one would hypothesize that chronic exposures to nanoparticles from hard copy devices could trigger development of chronic inflammation. These increased inflammatory and oxidative stress responses in humans were recapitulated in three human cell types exposed to the same particles - differentiated THP-1 macrophages, primary small airway bronchial cells, and primary nasal airway cells (Khatri et al., 2013b). Pulmonary inflammation was also observed in vivo in Balb/c mice (Pirela et al., 2013). It was further shown in THP-1 macrophages that photocopier nanoparticles induced increased transcription of inflammatory (TNF-α) genes, oxidative stress genes (HO-1, GPX1, SOD1), and apoptotic (p53, CASP8) genes (Khatri et al., 2013b). More recently, Pirela et al. (2016) used several cell lines (specifically THP-1 macrophages, small airway epithelial cells (SAEC), and lymphoblasts (TAC6)) exposed to a range of doses relevant to consumer exposures and found increased cellular toxicity plus increased expression of pro-inflammatory cytokines/chemokines and intracellular oxidative stress, as well as modest epigenetic modifications. Of note, we have shown in-vitro and in-vivo that copier nanoparticles are significantly more potent than micron-sized copier particles or 30 nm copper oxide nanoparticles, and comparable to or even more potent than welding fumes and diesel exhaust in inducing cytotoxicity, inflammatory markers, and lung injury (Pirela et al., 2014; Khatri et al., 2013b; Pirela et al., 2013; Khatri et al., 2013c).

There is understandably great interest in studying the effects of these nanoparticle exposures in the lower airways and other extra-pulmonary organs (as examples liver, kidneys, hematopoietic and nervous systems). Indeed, most of the existing studies on engineered nanoparticles in workplaces, or ultrafine air pollution, do focus on deep airways and circulatory markers (Liou et al., 2015; Liou et al., 2012). However, the upper airways are often overlooked. The cellular responses of the tissues in the upper airways to nanoparticles are of great interest for two major reasons. First, the upper airways are implicated directly in rhinitis, airway hyperreactivity, and other inflammatory conditions and diseases (Wagener et al., 2013). Secondly, the cells in the upper airways cross-talk with other cell types in the lower airways, exacerbating or sustaining inflammation (Wagener et al., 2013; Vroling et al., 2008; Bernstein et al., 2008; Bernstein and Smith, 2014). Of particular interest for human exposures to nanoparticles is the possible role of the olfactory bulb in nanoparticle uptake and translocation towards the brain. Anosmia, or loss of the sense of smell, and hyposmia (reduced smell sensation) are well-known conditions among photocopier operators, which historically has been attributed to ozone and volatile organic compounds (VOCs). However, this may not be the case, since technological innovations in the design of hard copy devices and the toner formulations have reduced both ozone and VOCs exposures (Kowalska and Zajusz-Zubek, 2009; Lee et al., 2001). Nanoparticles are now the major exposure source from hard copy devices and the main suspect agent.

Currently, the temporal dynamics of airway inflammation following chronic nanoparticle exposures, the exact molecular mechanisms involved in the cellular responses, and the fluctuations in levels of such inflammatory/injury markers under repeated, realistic exposures are poorly understood.

The main objective of this work is two-fold. First, the study aimed to establish that chronic exposures to nanoparticles from copy equipment can lead to chronic upper airway inflammation and chronic oxidative stress. We focused on markers of inflammation collected from nasal lavage and markers of oxidative stress in urine samples, in part because of the matched endpoints from acute human exposures and in-vitro studies. Second, the work aimed to better understand the within- and between-person variability in the expression levels of inflammatory biomarkers. This second aim of the study is helpful in gaining mechanistic insights into the mode of action of these nanoparticles, understanding inter-individual susceptibility, and in identifying optimal sampling schemes for large-scale human studies.

2. Methods

2.1. Study population and recruitment

We targeted full-time photocopy operators chronically exposed to nanoparticles from commercial photocopying as part of their job. Subjects were recruited by distributing flyers in commercial photocopy centers in the Northeast United States. Detailed presentations on the project were given for the interested individuals. Six operators from three commercial photocopying centers agreed to participate in the study. Eleven controls (individuals not involved with routine photocopying or printing activities, and with minimal exposure to nanoparticles from copying equipment) were also recruited at the same time. These controls were selected from employees in the same building as the photocopier operators and met the following criteria: