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Thirdhand smoke: Chemical dynamics, cytotoxicity, and genotoxicity in outdoor and indoor environments

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ABSTRACT

We tested the toxicity of thirdhand smoke (THS) using two controlled laboratory exposure scenarios and low levels of THS. One exposure modeled THS in a car parked outdoors, while the second modeled THS in a room without sunlight. The fabrics were exposed to cigarette smoke and then extracted in culture medium. Concentrations of nicotine, nicotine related alkaloids, and tobacco specific nitrosamines (TSNAs) were determined in fresh and aged extracts. The concentration of TSNAs increased with aging in the indoor experiment. THS extracts were used for cytotoxicity testing using mouse neural stem cells (mNSC), human dermal fibroblasts (hDF) and human palatal mesenchyme cells (hPM). Extracts from the car experiment inhibited mNSC proliferation in a live cell imaging assay and induced single strand DNA breaks in mNSC and hDF. In the indoor experiment, THS extracts made with medium containing serum proteins were significantly more toxic than extracts made with basal medium, and mNSC and hPM were more sensitive than hDF. These data indicate that: (1) aging of THS chemical differs on different fabrics and differs with and without sunlight; (2) very few cigarettes are sufficient to produce a toxic THS residue; and (3) protein enhances the efficiency of extraction of cytotoxic chemicals.

1. Introduction

While the adverse health effects of smoking and secondhand cigarette smoke exposure are well known (CDC, 2015; USDHHS, 2014), thirdhand smoke (THS) has only recently emerged as a public health concern (Matt et al., 2011). Experiments in cell-based systems and animal models are beginning to show that THS can be toxic. THS caused DNA damage in liver cells (Hang et al., 2013) and reduced neurite length and heart rate in zebra fish embryos (Hammer et al., 2011). In developing rat lung, 1-(N-methyl-N-nitrosamino)-1-(3-pyridinyl)-4-butanal) (NNA), a constituent of THS, disrupted signaling mechanisms by decreasing the levels of peroxisome proliferator-activated receptor and up-regulating fibronectin (Rehan et al., 2011). THS also produced detrimental effects on multiple organ systems in a mouse model (Martins-Green et al., 2014).

THS exposure can occur through inhalation, dermal contact, or ingestion. Toddlers and infants may have higher exposure to THS than adults because they are more likely to touch and mouth THS chemicals on toys, clothing, upholstery, and other indoor surfaces. Our recent work showed that THS remained on fabrics for many months after smoking had ceased (Bahl et al., 2014). In these experiments, we found significant levels of nicotine and tobacco specific nitrosamines (TSNAs), two of which (4-(methylnitrosamino_-1-(3-pyridyl)-1-butanone NNK) and N′-nitrosonornicotine (NNN)) are known carcinogens and the third, NNA, has been reported to cause DNA damage (Hang, 2010). NNA is not found in mainstream or secondhand tobacco smoke and is specific to THS (Sleiman et al., 2010). It is formed during aging of THS by reaction of nicotine with the ambient oxidant chemicals (Petrick et al., 2011; Sleiman et al., 2010). Chemical changes that occur in THS as it ages are likely to affect its mode of action and level of toxicity.

In the present study, we investigated the cytotoxicity and genotoxicity of THS using in vitro cell models and controlled laboratory conditions for the generation and collection of THS. We tested THS extracted from various fabrics on mouse neural stem cells (mNSC) from the neonatal

Abbreviations: ANOVA, analysis of variance; Cp, carpet; DMEM, Dulbecco's minimal essential medium; DMSO, dimethyl sulphoxide; DPBS, Dulbecco's phosphate-buffered saline; EDTA, ethylenediaminetetraacetic acid; EMEM, Eagle's minimal essential medium; FBS, fetal bovine serum; hDF, human dermal fibroblasts; hPM, human palatal mesenchyme cells; IC_{50}, concentration that produces a 50% response; LC–MS/MS, liquid chromatography coupled with tandem mass spectrometry; mNSC, mouse neural stem cells; MTT, [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]; NAB, *N*′-nitrosoanabasine; NAT, *N*′-nitrosoanatabine; NNA, 1-(N-methyl-N-nitrosamino)-1-(3-pyridinyl)-4-butanal); NNK, 4-(methylnitro-samino)-1-(3-pyridyl)-1-butanone; NNN, *N*-Nitrosonornicotine; PAH, polyaromatic hydrocarbon; PBS, phosphate-buffered saline; SC, seat cover; RT, room temperature; THS, thirdhand smoke; TSNAs, tobacco specific nitrosamines; VOC, volatile organic chemicals.

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cerebellum and on adult human dermal fibroblasts (hDFs). THS extracts from terry cloth were also tested with human palatal mesenchyme cells (hPM). The use of in vitro models for toxicity screening is rapid, can be predictive, and often serves as an excellent alternative to animal testing of environmental toxicants (Bahl et al., 2012; Behar et al., 2012a, 2012b; Eisenbrand et al., 2002; Talbot, 2008; Yu et al., 2006).

This study was carried out to test the hypotheses that low levels of THS adversely impact cell health and survival and that the chemicals in THS change as THS ages. We generated THS in two separate experiments. In one experiment, we exposed automotive seat cover fabric and automotive carpet samples to realistic concentrations of cigarette smoke in an acrylic chamber outdoors. The automobile experiment was designed to determine how THS behaves in an automobile parked outdoors for 1 month. This experiment was scaled to mimic a scenario in which 20 cigarettes are smoked per day for 30 days. In the second experiment, we exposed 100% cotton terry cloth to cigarette smoke in a controlled indoor exposure chamber with no sunlight. The smoke was generated at irregular intervals over 16 months. THS was extracted from fabrics in both experiments and analyzed using LC–MS/MS to determine the concentrations of nicotine, related alkaloids, and TSNAs. The MTT assay, which relies on conversion of a tetrazolium salt to a colored product through the enzymatic activity of metabolically active cells, was used to assay cell viability/survival and cytotoxicity. Rates of proliferation were measured using live cell imaging, and genotoxicity of THS extracts was examined using single cell gel electrophoresis (comet assay), which detects single strand breaks in DNA.

2. Materials and methods

2.1. Car simulation (outdoor) experiment

Synthetic car seat cover fabric (Auto Expressions part 5078760) and synthetic car carpet were purchased from O'Reilly Auto Parts (Riverside, CA) was exposed to puffs of sidestream cigarette smoke or puffs of indoor air (control) in an acrylic chamber measuring 32 cm × 32 cm × 60 cm and having a volume of 61,440 cm^3 (about 0.06 m^3), which is about 50 times smaller than the interior of an average car (3 m^3). An overview of the experimental design for the car experiment is shown in Fig. 1A.

The car exposure chamber was closed but the lid and the three ports were not sealed to allow some ventilation. Marlboro Red cigarettes were puffed using ISO standards (2 s puffs × 35 ml puff volumes for 1 min) on a smoking machine as described previously (Knoll and Talbot, 1998; Knoll et al., 1995) using a MasterFlex peristaltic pump (Barnart Company, Barrington, IL, Model #7520-00) to generate sidestream smoke which entered the chamber through polyvinylchloride tubing (Cole Parmer MasterFlex Tygon) for 4 min/day (1 min every 2 h for 8 h) over a total of 30 days (Fig. 1B). This is equivalent to about 20 cigarettes being smoked in a car/day, assuming it takes about 10 min for a person to smoke one cigarette. Control chambers were similar to experimental chambers but fabric was exposed to puffs of indoor air instead of cigarette smoke. The acrylic chambers were placed outdoors in an enclosed locked area (Fig. 1C), except when exposures were being done in the laboratory. During this phase of the experiment, the average highest daily

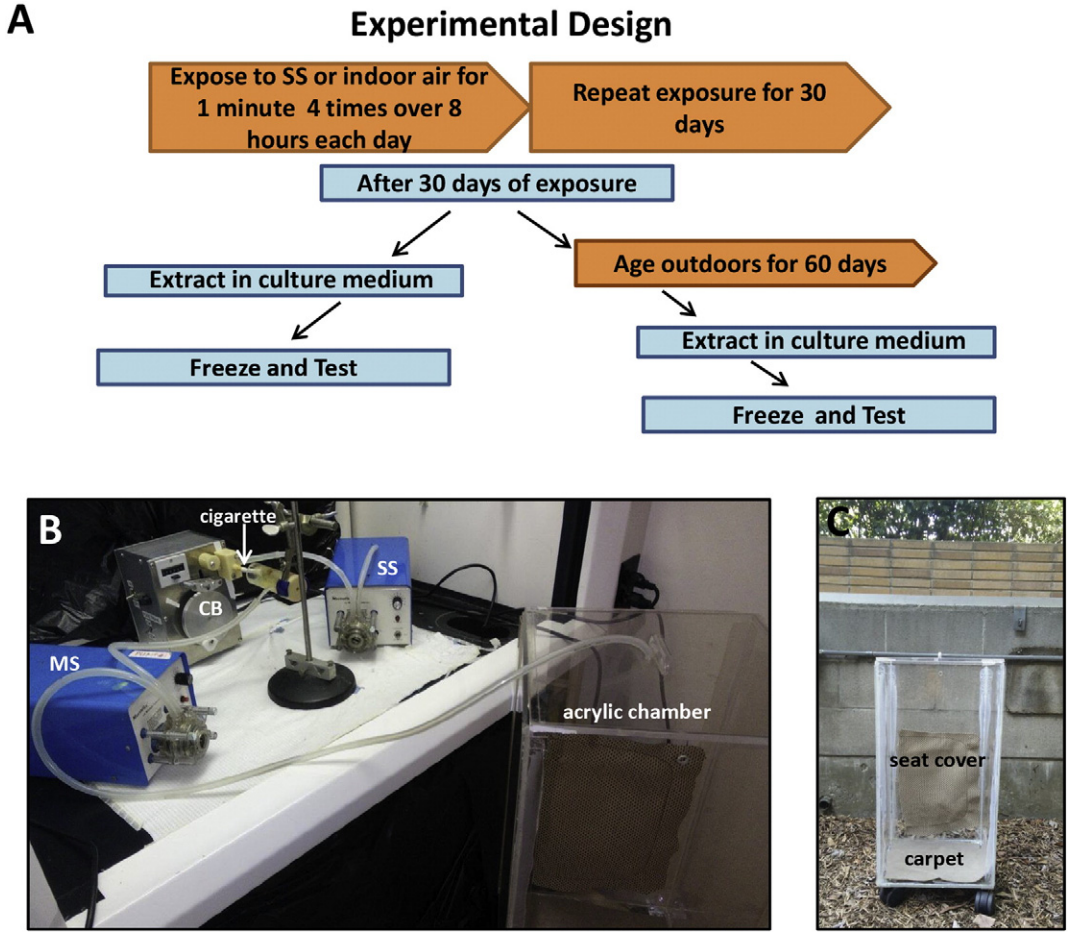

Fig. 1. Design and THS generation for the car experiment. (A). Over view of the design used for the car experiment. (B) THS generation set-up showing a Marlboro Red cigarette (arrow), two peristaltic pumps that were used for the generation of mainstream (MS) and sidestream smoke (SS), and the controller box (CB). SS or indoor air was introduced in to an acrylic chamber through tygon tubing and allowed to settle on car seat cover and car carpet in the chamber. SS smoke was generated for 1 min every 2 h, 4 times a day for 30 days. (C). Acrylic chamber with car seat cover and carpet in outdoor location to mimic a car.