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Children are particularly vulnerable to environmental tobacco smoke exposure: Evidence from biomarkers of tobacco-specific nitrosamines, and oxidative stress

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ABSTRACT

Background: Worldwide, smoking is a major public health problem, with exposure to environmental tobacco smoke (ETS) affecting both smokers, and passive smokers, including children. Despite ETS also describing secondhand, and thirdhand smoke (SHS, and THS respectively), the health effects of exposure to passive smoking via these sources are not fully understood, particularly in children. Although cotinine, the primary proximate metabolite of nicotine, has been widely used as a biomarker of ETS exposure, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), the metabolite of the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), provides a uniquely important contribution, both as a biomarker of exposure, and as a specific risk indicator for pulmonary carcinogenesis.

Methods: We used LC-MS/MS to study NNK metabolites, cotinine, and 8-oxo-7,8-dihydro-2′-deoxyguanosine (a biomarker of oxidative stress), in the urine of 110 non-smoking adults (age range: 23–62) and 101 children (age range: 9–11), exposed to ETS.

Results: In our study of passive smoking adults, and children exposed to ETS, we showed that although the children had a similar urinary level of cotinine compared to the adults, the children had approximately two times higher levels of urinary total NNAL ($P = 0.002$), and free NNAL ($P = 0.01$), than adults. The children also had three times lower ability to detoxify NNK than adults ($P < 0.001$). Furthermore, the children showed 1.5 times higher ratio of total NNAL/cotinine than adults ($P = 0.01$), implying that THS is another important source of ETS in this population. Furthermore, ETS exposure in children appeared to lead to an increase in levels of oxidative stress.

Conclusions: Taken together, our results demonstrate that, in children, THS may play an important role in the ETS exposure, and that children are at particular risk of ETS-induced health effects.

1. Introduction

Environmental tobacco smoke (ETS) exposure, or "passive smoking", is a significant, and preventable, cause of disease and disability, and an important public health problem (Oberg et al., 2011). ETS includes secondhand smoke (SHS) and thirdhand smoke (THS) and

Abbreviations: 8-oxodGuo, 8-oxo-7,8-dihydro-2′-deoxyguanosine; Cr, creatinine; ESI, electrospray ionization; ETS, environmental tobacco smoke; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LOD, limit of detection; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NNAL-Gluc, glucuronidated NNAL; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; SHS, secondhand smoke; SPE, solid phase extraction; THS, thirdhand smoke; TSNA, tobacco-specific nitrosamine

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comprises therefore any tobacco smoke exposure outside of active smoking (Protano and Vitali, 2011). ETS increases the risk of lung cancer and heart disease in adults who do not smoke, and in children it also increases the risk of sudden infant death syndrome, asthma, bronchitis, and pneumonia (Cao et al., 2015).

SHS is a mixture of smoke emitted from the burning of tobacco products, and the smoke exhaled from smokers. SHS, the third leading cause of preventable death worldwide, contains > 7000 chemicals, including about 70 known and probable carcinogens, toxicants and irritants (Rodgman and Perfetti, 2013), as well as inducers of oxidative stress (Varela-Carver et al., 2010), which is strongly implicated in carcinogenesis (Olinski et al., 2018). Although some of the chemicals present in SHS remain predominantly in the gas phase, and can be removed by ventilation, a significant fraction adheres to the clothing, skin and hair of smokers, and to surfaces, furnishings, and dust in indoor environments, persisting for a longer time; this is considered THS (Matt et al., 2017). Indoor environments, in which tobacco is regularly smoked, become reservoirs of THS that store and gradually release pollutants over time, potentially leading to the involuntary exposure of non-smokers through inhalation, ingestion and dermal transfer, long after smoking has taken place (Matt et al., 2017).

Despite worldwide initiatives to decrease tobacco smoking, it is estimated that as much as 40% of children (< 14 years old) worldwide are still exposed to tobacco smoke (Oberg et al., 2011). Children are more sensitive than adults to ETS for several reasons, including immaturity of immune systems, and smaller bronchial tubes; they also breathe faster, and hence inhale more harmful chemicals per kg of body weight than adults (Hang et al., 2017; Jenabian et al., 2015). For the reasons noted above, via THS, children may be exposed to the harmful chemicals in tobacco smoke, even if parents avoid smoking in their presence (de la Riva-Velasco et al., 2012). THS is a new concept in the field of tobacco control, and there are insufficient data on its greater effects on children compared to adults (Dede and Cinar, 2016). Indeed, THS could be particularly dangerous for children who spend much of their time indoors, on hands and knees, touching, tasting, putting fingers in their mouths, playing on floors and in contact with contaminated surfaces (Winickoff et al., 2009).

Nicotine is the major alkaloid of tobacco, and is responsible for tobacco addiction. Cotinine, the major metabolite of nicotine, is widely used as a biomarker of tobacco smoke exposure (Avila-Tang et al., 2013). Tobacco specific nitrosamines (TSNAs) are a group of carcinogens, as the name suggests, present in tobacco; an example of which is 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), which is an important inducer of lung adenocarcinoma. NNK is formed primarily during tobacco curing, when nicotine or pseudo-oxynicotine reacts with nitrite in tobacco (Hecht, 1998). In vivo, NNK is converted metabolically to 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), which is also a potent pulmonary carcinogen (Hecht, 2008). NNAL can be further metabolized through glucuronidation to form glucuronidated NNAL (NNAL-Gluc), that is readily excreted in urine along with free NNAL. It has been suggested that NNAL may have a greater public health impact and better predictive utility for the adverse health effects of ETS exposure, compared to cotinine (Avila-Tang et al., 2013).

Given that NNK is believed to contribute to the cancer risk in tobacco smoke-exposed populations, and that oxidative stress is implicated in the tobacco-related carcinogenesis, it would seem logical to study both to better understand the role tobacco chemicals play in influencing health both in childhood and beyond (Peterson and Hecht, 2017). However, limited data are available in the literature on NNK metabolites and oxidative stress in children exposed to ETS. In the present study, we quantified cotinine, free NNAL, total NNAL (free NNAL plus NNAL-Gluc) and a biomarker of oxidative stress, 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodGuo) (Hu et al., 2015; Olinski et al., 2018), in the urine of children of elementary school age, and adults. To the best of our knowledge, this is the first study to assess NNK detoxification in both children, and passive smoking adults, and to also investigate the influence of ETS exposure on oxidative stress in children and adults.

2. Experimental

2.1. Chemicals

All solvents and salts were of analytical grade. Reagents were purchased from the indicated sources: NNK, NNAL, d_3-NNK and d_3-NNAL from Toronto Research Chemicals (Ontario, Canada); 8-oxodGuo, cotinine, β-glucuronidase (type IX-A, *Escherichia coli*) from Sigma-Aldrich (MO, USA); d_3-cotinine from Cerilliant (TX, USA); $^{15}N_5$-8-oxo-7,8-dihydro-2′-deoxyguanosine ($^{15}N_5$-8-oxodGuo) from Cambridge Isotope Laboratories (MA, USA).

2.2. Participants and urine samples

This study was approved by the Institutional Review Board of Chung Shan Medical University Hospital. A total of 113 apparently healthy adults who reported ETS exposure at work place, or at home, were recruited. Written informed consent was obtained from each adult participant, and a spot urine sample then collected. For the recruitment of school-aged children, all families received an initial contact letter from the principal of their child's school describing the study, and the opportunity for participation. Following expressions of interest, a trained recruiter then obtained written consent from each child's parents, and administrated a questionnaire to the children to collect the information about ETS exposure at home. The questions included: (a) “does anyone smoke when you are at home?”, (b) “how many smokers live together in your home?” and (c) how many hours per day you spent at home in the past 7 days? There were 273 children (age range: 9–11) who agreed to volunteer (all with parental consent). Of these 273 children, a total of 129 had the positive answers (“yes” and/or ≥1 smokers) to the question (a) and (b), and were considered exposed to ETS. We also asked about “did you take medicine or daily vitamin supplement within the past 7 days?”. Of these 129 children, 28 children also reported a regular use of vitamin supplement (e.g., vitamin C) or the use of medicine, and were excluded from the study. A spot urine sample was collected from the remaining 101 children, under the supervision of the school nurses. Urine samples were collected in 50 mL polypropylene containers, kept at 4 °C during sampling, and then stored at −20 °C prior to analysis.

2.3. LC-MS/MS analysis of urinary NNK metabolites

Urine samples were thawed, vortexed, and warmed to 37 °C for 10 min to release possible NNK and NNAL from precipitate, followed by centrifugation at 5000*g* for 5 min. For free NNAL determination, 1 mL of urine was spiked with 50 μL a solution containing 0.5 ng each of d_3-NNK and d_3-NNAL as internal standards in deionized water. Because the urinary level of NNK/NNAL in passive smokers can be extremely low, the urine sample was pre-concentrated by manual solid phase extraction (SPE). The urine mixture was then loaded onto a Sep-Pak C18 cartridge (100 mg/1 mL, Waters, MA, USA), preconditioned with 1 mL methanol and 1 mL deionized water. The cartridge was then washed with 1 mL of 5% (v/v) methanol containing 1 mM ammonium acetate (AA), and eluted with 1 mL of 40% (v/v) methanol containing 1 mM AA. The eluate was dried under vacuum and redissolved in 0.1 mL of 5% (v/v) methanol containing 1 mM AA ready for online SPE LC–MS/MS analysis.

For total NNAL (free NNAL plus NNAL-Gluc) determination, 0.5 mL of urine was spiked with 50 μL solution containing 0.5 ng each of d_3-NNK and d_3-NNAL, and then hydrolyzed with 500 μL of β-glucuronidase (2000 U/mL) in 75 mM phosphate buffer (pH 6.8) at 37 °C for 24 h in the dark, to convert NNAL-Gluc to NNAL. After incubation, the urine sample was pre-concentrated using manual SPE, as described