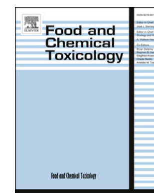
Food and Chemical Toxicology 106 (2017) 147–154

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox



Brand variation in oxidant production in mainstream cigarette smoke: Carbonyls and free radicals

Samantha M. Reilly [a], Reema Goel [a], Neil Trushin [a], Ryan J. Elias [b], Jonathan Foulds [a], Joshua Muscat [a], Jason Liao [a], John P. Richie Jr. [a, *]

[a] *Department of Public Health Sciences, Pennsylvania State University Tobacco Center of Regulatory Science (TCORS), Pennsylvania State University College of Medicine, Hershey, PA, United States*

[b] *Department of Food Science, Pennsylvania State University, College of Agricultural Sciences, University Park, PA, United States*

ARTICLE INFO

Article history:
Received 26 April 2017
Received in revised form
15 May 2017
Accepted 17 May 2017
Available online 18 May 2017

Keywords:
Acetaldehyde
Formaldehyde
Crotonaldehyde
Methyl ethyl ketone
Propionaldehyde
Oxidants
Tobacco smoke

ABSTRACT

Oxidative stress/damage resulting from exposure to cigarette smoke plays a critical role in the development of tobacco-caused diseases. Carbonyls and free radicals are two major classes of oxidants in tobacco smoke. There is little information on the combined delivery of these oxidants across different cigarette brands; thus, we set out to measure and compare their levels in mainstream smoke from popular US cigarettes. Mainstream smoke from 28 different cigarette brands produced by smoking (FTC protocol) was analyzed for five important, abundant carbonyls, and levels were compared to previously determined free radical for the same brands. Overall, there were large variations (3- to 6-fold) in carbonyl levels across brands with total carbonyl levels ranging from 275 to 804 μg/cigarette, which persisted even after adjusting for ventilation. Individual carbonyl levels were highly correlated with each other (r^2: 0.40−0.95, $P < 0.003$) except for formaldehyde. Both gas-phase (r^2: 0.37, $P = 0.006$) and particulate-phase (r^2: 0.27, $P = 0.005$) free radicals were correlated to total carbonyl content; however, this correlation disappeared after adjusting for ventilation. These data show that overall oxidant production varies widely by cigarette brand and the resulting difference in oxidant burden could potentially lead to differences in disease risk.

© 2017 Elsevier Ltd. All rights reserved.

1. Introduction

Cigarette smoking is the number one cause of preventable death worldwide, causing one of every five deaths in the United States (U.S.) each year (Danaei et al., 2009; U.S. Department of Health and Human Services, 2014; Xu et al., 2015). It leads to lung cancer (Doll and Hill, 1950; Wynder and Graham, 1950), chronic obstructive pulmonary disease (COPD) (Burney et al., 2014; Zaher et al., 2004), cardiovascular and many other diseases (Krupski, 1991; U.S. Department of Health and Human Services, 2014). Oxidative stress is thought to play a major role in the development of many of these tobacco-caused diseases (Church and Pryor, 1985; Pryor, 1997); thus, it is important to better understand the total oxidant burden resulting from cigarette smoke in order to gain a better assessment of a smoker's potential oxidative risk.

While carbonyls are most known for their toxic and carcinogenic effects (U.S. Food and Drug Administration, 2012), carbonyls are also a major class of powerful oxidants, depleting glutathione (Wooten et al., 2006), forming adducts with DNA bases (Halliwell and Gutteridge, 2015), and producing free radicals when metabolized (Kundu et al., 2007). Together, these effects have linked carbonyls to a significant number of smoking-related diseases (U.S. Food and Drug Administration, 2012). Previous reports on carbonyls in cigarette smoke show their levels vary by brand (Bodnar et al., 2012; Counts et al., 2004, 2005; Ding et al., 2015; Hammond and O'Conner, 2008; Marcilla et al., 2012; Roemer et al., 2003). Potential sources of variation between cigarette brands include differences in ventilation, tobacco type, filter design, additives, and paper porosity as well as a smoker's unique puff profile (Baker et al., 2004a,b; Chen et al., 2014; Dittrich et al., 2014; Hoffmann et al., 1995; Roemer et al., 2012; Seeman et al., 2003). While carbonyl

Abbreviations: TPM, total particulate matter; CV, coefficient of variation.

* Corresponding author. Department of Public Health Sciences, Pennsylvania State University Tobacco Center of Regulatory Science (TCORS), Pennsylvania State University College of Medicine, 500 University Dr. – Mail Code: CH69, Hershey, 17033, PA, United States.

E-mail address: jrichie@psu.edu (J.P. Richie).

http://dx.doi.org/10.1016/j.fct.2017.05.035
0278-6915/© 2017 Elsevier Ltd. All rights reserved.

production is known to differ by brand, little is known as to how this variation may compare to the other major class of oxidants, free radicals. Thus, we sought to determine the variation in the levels of five important and abundant tobacco smoke carbonyls (formaldehyde, acetaldehyde, propionaldehyde, crotonaldehyde, and methyl ethyl ketone (MEK)) and compare their levels to those of free radicals. Since both classes of oxidants are produced by the incomplete combustion and pyrolysis of the tobacco in the cigarette (U.S. Department of Health and Human Services, 2014), we hypothesize that these levels will vary similarly amongst brand/type. Altogether, this information can be used to better understand the total oxidant burden of cigarette smoke. To this end, we recently developed standardized methodology to analyze both the highly reactive gas-phase free radical content and the stable particulate-phase free radical content of cigarette smoke and applied the method to quantify the free radical levels in 27 brands of popular US cigarettes for the first time (Goel et al., 2017). In the current paper, we report on the levels of carbonyls in the same 27 cigarette brands smoked under identical conditions (FTC protocol) and use these results to determine the relationship of these two oxidant classes across cigarette brands.

2. Materials and methods

2.1. Cigarettes

Twenty-seven cigarette brands (all king-sized except for Virginia Slims Gold) were purchased from local retailers, chosen for their popularity in the local area (Dauphin and Lebanon Counties, PA) based on self-report from the retailers and their combined share of the US cigarette market (these brands represent approximately 70% of the US market) (Sharma et al., 2015). The 3R4F research cigarette was obtained from the University of Kentucky (Lexington, Kentucky, USA) and was used as a reference cigarette. The cigarettes were stored at −20 °C in airtight plastic bags.

2.2. Materials

Acetonitrile (ACN) and concentrated hydrochloric acid (12N HCl) were purchased from Fisher Scientific (Pittsburgh, PA, USA) and used as supplied. Diglyme and dinitrophenylhydrazones of formaldehyde, acetaldehyde, crotonaldehyde, propionaldehyde, and MEK were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used as supplied. 2,4-Dinitrophenylhydrazine (DNPH) was purchased from BOC Sciences (Shirley, NY, USA) and recrystallized before use to remove water, which can affect the reactivity of DNPH with carbonyls (Risner and Martin, 1994). Recrystallization was done by dissolving 17 g DNPH in 350 mL acetonitrile, heating this solution at 70 °C for 60 min, and then cooling to room temperature. The crystals were collected via vacuum filtration, and then stored in a desiccator.

2.3. Mainstream smoke generation

The cigarettes were conditioned for testing by removing them from cold storage and placing them in a constant humidity chamber (60% relative humidity, 22 ± 1 °C), for at least 48 h before smoking. Mainstream smoke was generated by a 30-port smoking machine (Jaeger-Baumgartner, CSM JB2080). Five cigarettes were smoked simultaneously on the machine under a FTC smoking protocol: 35 mL puff volume, 2 s puff duration, and 60 s puff interval. We tested for breakthrough, which is when any carbonyl passes through the capturing solution and thus is not captured, by adding a second impinger in line. Breakthrough was minimal (~2%) for all carbonyls when testing between one and five cigarettes on the smoking machine, but did become an issue when testing ten or more cigarettes. Thus, we limited our study to five or less cigarettes per collection.

2.4. Derivatization of carbonyls

DNPH solution was made as described previously (Risner and Martin, 1994) by dissolving 1.0 g recrystallized DNPH in a mixture of 50 mL diglyme, 360 μL 12N HCl, and 150 mL ACN Mainstream smoke generated from five cigarettes of each brand as described above was passed directly into an impinger containing 10 mL of DNPH solution placed after the pump. The sample was then transferred into a 20 mL scintillation vial and stored at 4 °C until HPLC-UV analysis, which was performed within 5 days of collection. We performed a minimum of two replicates of each collection (n = 2–4 for all experiments). Day to day assay variation was low (Coefficient of variation (CV) = 5%) for total carbonyls in 3R4F cigarettes (Table 1). When the impinger was moved upstream of the pump, yields were increased for acetaldehyde only (15%; p-value: 0.04); however, it was difficult to maintain a consistent flow rate in this configuration, so it was not used for further experiments.

2.5. HPLC-UV analysis

High performance liquid chromatography with ultraviolet detection (HPLC-UV) analyses were performed using a binary system consisting of two Waters (Milford, MA, USA) 510 pumps, a Waters 440 UV absorbance detector, and a Hitachi (Tokyo, Japan) D-2500 Integrator. Compounds were separated using a Bondclone C18 column (10 μm × 300 mm x 3.9 mm; Phenomenex, Torrance, CA, USA) using two mobile phases: water (A) and acetonitrile (B). The elution gradient parameters are: 0 min, 90% A, 10% B; 20 min, 10% A, 90% B; 25 min, 10% A, 90% B, 27 min, 90% A, 10% B; and 37 min. 90% A, 10% B. The flow rate is 1.0 mL/min for all time steps, and the detection wavelength was 254 nm. All sample injections were 10 μL and were manually injected. All measurements were carried out at room temperature (22 ± 1 °C). Supplementary Fig. 1 is a representative chromatogram. This method was found to have good precision (CV: 6–12%) for all carbonyls tested, which was determined by 12 replicate injections of a collected cigarette sample. Accuracy was determined through spiking a sample with known amounts of carbonyl standards and found to have a CV of 4%.

2.6. GC-MS analysis

All peaks, except crotonaldehyde, were verified by collecting the eluting peaks from the HPLC-UV and analyzing by GC-MS. To do this, an Agilent Technologies 7890A gas chromatograph (Agilent Technolgies, Inc., Santa Clara, CA) fitted with a Gerstel MPS2 autosampler (Gerstel GMbH & Co. KG. Mülheim an der Ruhr, Germany) was used in combination with an Agilent 5975C mass selective detector. The instrument and autosampler were controlled by Agilent MassHunter GCMS Acquisition software (Version B.07.00 SP2.1654) and Gerstel Maestro 1 software (Version1.2.20.41), respectively. The GC was fitted with a J&W VF-35ms capillary column with dimensions 60 m × 0.25 mm x 0.25 μm. The inlet was fitted with a borosilicate glass single taper inlet liner with wool (6.5 mm o.d., 4.0 mm i.d., 78.5 mm long) (Restek, Bellefonte, PA). Helium (ultra-high purity) was used as the carrier gas at a constant flow rate of 1.4 mL/min. Depending upon analyte concentration within the sample, between one and 3 μL of sample were introduced to an injector operated in split-less mode and maintained at a temperature of 270 °C. The splitter was opened to 71:1 after 60 s. The GC oven was first held at 50 °C for 2.5 min, then increased to

Download English Version:

https://daneshyari.com/en/article/5560116

Download Persian Version:

https://daneshyari.com/article/5560116

Daneshyari.com