Toxicology and Applied Pharmacology 236 (2009) 270–275

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

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

Longitudinal distribution of ozone absorption in the lung: Comparison of cigarette smokers and nonsmokers

Melissa L. Bates [a,*], Timothy M. Brenza [b], Abdellaziz Ben-Jebria [a,b], Rebecca Bascom [c], James S. Ultman [a,b]

[a] Interdisciplinary Graduate Degree Program in Physiology, Pennsylvania State University, University Park, PA 16802, USA
[b] Department of Chemical Engineering, Pennsylvania State University, University Park, PA 16802, USA
[c] Division of Pulmonary, Allergy and Critical Care Medicine, Penn State College of Medicine, Hershey, PA 17033, USA

ARTICLE INFO

Article history:
Received 16 June 2008
Revised 26 January 2009
Accepted 10 February 2009
Available online 20 February 2009

Keywords:
Ozone
Cigarette
Smoker
Distribution
Uptake
Pulmonary function
Spirometry
Capnogram
Bolus
Longitudinal
Alveolar plateau
Expirogram
Uptake
Lung

ABSTRACT

In nonsmokers, ozone (O_3) is removed primarily by the epithelial lining fluid (ELF) of the conducting airways. We hypothesized that cigarette smokers, whose ELF antioxidant capacity may be limited by smoking, would remove less O_3 from their conducting airways than nonsmokers. We recruited 29 nonsmokers (17M, 12F) and 30 smokers (19M, 11F, 4 ± 4 pack-years) with similar anthropometric characteristics and measured the longitudinal distribution of O_3 using the bolus inhalation method. We also assessed the physiological effect of this transient exposure regimen using forced spirometry and capnography. Contrary to our hypothesis, the penetration volume at which 50% of a bolus was absorbed was not different between smokers and nonsmokers (97.1 ± 5.4 mL versus 97.9 ± 5.8 mL, $p = 0.92$). However, smokers did experience an increase in the slope of the alveolar plateau of the capnogram (S_N) ($8.1 \pm 3.2\%$, $p = 0.02$) and a small decrease in FEV_1 ($-1.3 \pm 0.6\%$, $p = 0.03$), whereas nonsmokers did not ($\Delta FEV_1 - 0.1 \pm 0.5\%$ and $\Delta S_N - 0.2 \pm 2.5\%$, $p > 0.10$). Thus, smokers are more sensitive to inhaled O_3 boluses than nonsmokers, despite a similar internal dose distribution.

Introduction

Ozone (O_3), at the ground level, is an oxidant gas that makes a major contribution to photochemical smog. Short-term exposure causes alterations in pulmonary function, such decrements in forced expiratory volume in 1 s (FEV_1) and increases in specific airway resistance, as well as lung inflammation (Beckett et al., 1985, Horstman et al., 1990, Mudway et al., 2000, Adams, 2002). Longer term exposure can affect lung growth in children and potentially permanent lung damage in adults (Kunzli et al., 1997, Frischer et al., 1999). Therefore, understanding the underlying dosimetric and toxicological processes is important in predicting the degree to which a population may experience health effects from O_3 exposure.

The epithelial lining fluid (ELF) consists of the mucous blanket in the conducting airways and the surfactant-rich aqueous layer in the respiratory airspaces. Ozone is removed from the gas phase by simultaneous diffusion into the ELF and chemical reaction with antioxidants and other resident substrates (Samet and Cheng, 1994, Postlethwait and Ultman, 2001). In nonsmokers inhaling at flow rates similar to those experienced during quiet breathing or light to moderate exercise, removal of O_3 occurs primarily in the conducting airways (Hu et al., 1994). Cigarette smoking may, however, decrease the uptake rate of O_3 into the conducting airways in two ways: first, it may increase the resistance to diffusion by inducing mucous hypersecretion that thickens the ELF; second, it may directly depress the antioxidant content of the ELF by oxidation (Rahmann and MacNee, 1999, Samet and Cheng, 1994). As a result of reduced uptake of O_3 into conducting airways, cigarette smoking may shift the longitudinal distribution of O_3 uptake distally towards the respiratory airways, thereby affecting the pulmonary gas exchange process.

We previously developed the bolus inhalation technique to measure the longitudinal distribution of O_3 uptake in the respiratory tract (Ben-Jebria et al., 1991). This method has been proven useful to investigate the effect of flow rate and pre-continuous exposure to O_3, SO_2 and NO_2 on the longitudinal distribution of O_3 in healthy nonsmokers (Hu et al., 1994, Rigas et al., 1997, Bush et al., 1996, Asplund et al., 1996). In the current study we compared O_3 distributions in a group of well-matched of healthy smokers and

* Corresponding author. Department of Kinesiology, The University of Wisconsin, 2039 Natatorium, 2000 Observatory Drive, USA. Fax: +1 608 262 1656.
E-mail address: mlbates@wisc.edu (M.L. Bates).

0041-008X/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.taap.2009.02.006

nonsmokers (height, weight, age, sex, pulmonary function) to test the hypothesis that smoking increases the penetration depth of O_3. The effect of brief O_3 bolus exposures on pulmonary function has never been studied in a systematic manner. Thus, we further postulated that as a result of distribution differences, smokers would experience greater changes in markers of pulmonary gas exchange than nonsmokers. Specifically, we measured FEV1, and from capnograms, determined the Fowler dead space volume (V_D) and the normalized slope of the alveolar plateau (S_N) before and after bolus exposure in both smokers and nonsmokers. Our V_D and S_N measurements have a unique feature in that they are independent indicators of proximal and distal lung function, respectively.

Methods

Subject population. Twenty-nine nonsmokers (17 men and 12 women) and thirty smokers (19 men and 11 women) were recruited from the student and staff population of the Pennsylvania State University and from the surrounding community. All screening and research procedures were carried out in the General Clinical Research Center and were approved by the Office of Research Protections at the Pennsylvania State University.

After reviewing information about the study, participants completed an informed consent and were scheduled for a screening session during which they completed medical and smoking history questionnaires and a standard clinical spirometry test to measure FEV_1 and forced vital capacity (FVC). Blood was drawn to determine cardiac risk and to verify smoking history by plasma cotinine analysis. Furthermore, participants received a physical exam, ECG, and exercise tolerance test to verify the lack of apparent cardiovascular disease. Smokers were admitted into the study if they reported a smoking history of greater than one pack-year and daily cigarette use. Nonsmokers were admitted if they reported a smoking history of <0.5 pack-years and no tobacco use within the last three years. Additional inclusion criteria for both nonsmokers and smokers included an $FEV_1/FVC>0.7$ and $FEV_1>80\%$ using Knudsen predicted values (Knudsen et al., 1976), no history or presentation of cardiovascular or respiratory disease, and no regular use of medication, except birth control pills. Female participants were given a urine pregnancy test before the screening and bolus exposure sessions and excluded if pregnant or breast-feeding.

Ozone distribution. Volunteers participated in a research session lasting approximately 2 h during which the distribution of ozone within their respiratory tract was measured using methods previously described in detail (Hu et al., 1994). Although smokers were not asked to abstain from tobacco use before the session, a minimum of 45 min elapsed between the participant's arrival and the first test measurement. While breathing through a mouthpiece, the participant initiated two to three bolus test breaths per minute, controlling respiratory flow at 1.0 L/s. A 20-mL bolus of ozonated air was injected into each test breath, and the timing of the injection was varied by the experimenter until 60–80 breaths ranging from penetration volumes of 40 and 300 mL had been recorded for further analysis. Breaths that did not fall within 15% of the targeted 1.0 L/s flow were discarded. Throughout each bolus test breath, respiratory flow rate and O_3 concentration were continuously monitored just proximal to the mouth. Absorbed O_3 fraction (Λ) was calculated as 1 minus the ratio of the integrals of the inspired and expired O_3 concentration–volume curves. Penetration volume (V_P) was computed as the mean volume of the inhaled O_3 concentration–volume curve relative to the end of inspiration (see Fig. 1).

In order to smooth these data, the Λ values were separated into 20 mL increments (*i.e.,* bins) of V_P. After averaging the Λ values within each bin for each participant, the bin averages were pooled and averaged for each population of interest. Both V_P and V_D were corrected for the equipment dead space and the non-absorbing portion of the upper airways such that $V_P = 0$ reflects the first point at which Λ rose above zero. To account for the possible effect of differences in conducting airway volume between the two populations, V_P was normalized by the participant's pre-exposure value of V_D. Values for Λ for each individual participant were separated into V_P/V_D bins of 0.2 and were averaged in the same manner as the non-normalized data. As a single parameter with which to describe the Λ–V_P distribution, a V_{P50} for each individual was calculated by averaging the five V_P values immediately above and the five V_P values immediately below the point at which Λ equaled 0.5.

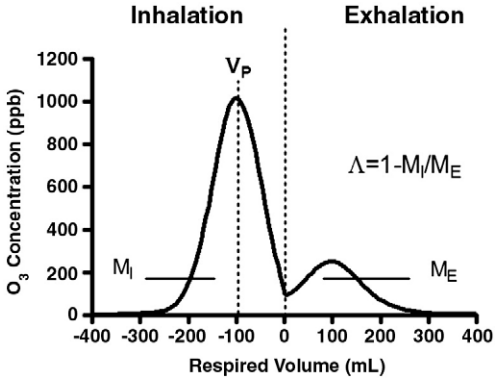

Fig. 1. Concentration of an O_3 bolus as a function of respired volume. M_I and M_E represent the inhaled and exhaled dose of O_3, calculated by integrating the inhaled and exhaled concentrations as a function of volume. The penetration volume (V_P) is indicated as the midpoint of the inspiratory concentration profile relative to the end of inspiration.

Measurement of lung responses. Before and immediately after the bolus inhalation experiment, values of FEV_1 and FVC were determined using a clinical spirometer (KoKo Model, Ferraris). Spirometry was performed according to the 2005 American Thoracic Society guidelines (Miller et al., 2005). A minimum of three maneuvers were performed and maneuvers were repeated until the two largest values of FEV_1 and FVC were within 0.15 L of each other. Capnograms were obtained by continuously monitoring respired CO_2 concentration and respired air flow using an apparatus described by Taylor et al. (2006). Beginning at functional residual capacity, participants completed two sequential breaths while controlling their respiratory air flow at 250 mL/s. The first breath consisted of 750 mL inhaled and exhaled volumes. The second breath consisted of a 750 mL inhalation and an exhalation of at least 1250 mL. The Fowler dead space (V_D) and slope of the alveolar plateau were calculated from the second expiration using the method described by Taylor et al. In order to minimize breath-to-breath variability in exhaled CO_2 concentration, S_N was normalized by the amount of CO_2 in the expired breath, as determined from the integral of the expired CO_2 concentration–volume record.

Verification of smoking status. Smoking status was verified both by questionnaire and by assaying for plasma cotinine. Cotinine was quantified via high performance liquid chromatography (HPLC) using the following variation of a method described by Ghosheh et al. (2000). Blood samples were drawn into heparin containing tubes, centrifuged, and the plasma layer stored at −77 °C. At the time of analysis, the plasma sample was thawed, treated with NaOH, extracted with dichloromethane, and evaporated. The resulting residue was dissolved in deionized, distilled water and analyzed by HPLC employing UV detection at 200 nm, tandem LC-8, LC-18 and cation-exchange columns and an isocratic mobile phase of 0.3M ammonium phosphate buffer:water:methanol (15:70:15). LC-18 and L-8 columns were added to Crook's method and a detection wavelength of 200 nm employed in order to improve the resolution of cotinine from a