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Effects of biomass burning on nasal mucociliary clearance and mucus properties after sugarcane harvesting

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ABSTRACT

Objective: Biofuel from sugarcane is widely produced in developing countries and is a clean and renewable alternative source of energy. However, sugarcane harvesting is mostly performed after biomass burning. The aim of this study was to evaluate the effects of harvesting after biomass burning on nasal mucociliary clearance and the nasal mucus properties of farm workers.
Methods: Twenty seven sugarcane workers (21–45 years old) were evaluated at the end of two successive time-periods: first at the end of a 6-month harvesting period (harvesting), and then at the end of a 3-month period without harvesting (non-harvesting). Nasal mucociliary clearance was evaluated by the saccharine transit test, and mucus properties were analyzed using *in vitro* mucus contact angle and mucus transportability by sneeze. Arterial blood pressure, heart rate, respiratory rate, pulse oximetry, body temperature, associated illness, and exhaled carbon monoxide were registered.
Results: Data are presented as mean values (95% confidence interval). The multivariate model analysis adjusted for age, body-mass index, smoking status and years of working with this agricultural practice showed that harvesting yielded prolonged saccharine transit test in 7.83 min (1.88–13.78), increased mucus contact angle in 8.68 degrees (3.18–14.17) and decreased transportability by sneeze in 32.12 mm (-44.83 to -19.42) compared with the non-harvesting period. No significant differences were detected in any of the clinical parameter at either time-period.
Conclusion: Sugarcane harvesting after biomass burning negatively affects the first barrier of the respiratory system in farm workers by impairing nasal mucociliary clearance and inducing abnormal mucus properties.

1. Introduction

Biofuel production has been proposed as a clean and renewable alternative energy source because it is less polluting than exhaustible fossil fuels. Brazil is one of the leading countries in the production of ethanol derived from sugarcane due to a government program promoting biofuel production that was initiated in the 1970s. Sugarcane plantations cover approximately seven million hectares of Brazilian land, and they surround many cities. In addition to reducing the use of fossil fuels, ethanol production increases farm job opportunities in poor areas (Goldemberg, 2007). However, one of the major drawbacks of this method of biofuel production is that sugarcanes are typically burned prior to being cut and harvested manually. This rudimentary agricultural practice (biomass burning) is performed primarily to facilitate sugarcane harvesting by eliminating dry sugarcane straws, dead growth, and venomous animals, such as vipers and scorpions (Uriarte et al., 2009; Mazzoli-Rocha et al., 2008). There is mounting evidence that biomass burning is a significant contributor to the atmospheric release of toxic gases and total suspended particles throughout the world (Torres-Duque et al., 2008).

Conjunctivitis, exacerbation of respiratory symptoms, increased numbers of emergency room and hospital admissions, and increased mortality in patients with chronic cardiorespiratory diseases have

Abbreviations: COPD, chronic obstructive pulmonary disease; PM, particulate matter

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been described in populations living in metropolitan areas (Brunekreef and Holgate, 2002; Brook et al., 2004; WHO, 2005; Larrieu et al., 2009) and in municipalities near locations where biomass burning occurs (Cançado et al., 2006; Arbex et al., 2007; Uriarte et al., 2009). Sugarcane biomass burning is performed every evening throughout the harvesting season in areas that will be harvested the following day. The daily routine of these farm workers requires exposure to areas with high concentrations of suspended particles and elevated temperatures due to the previous burning process. Over a long workweek of 8–9 h per day and 5 shifts per week, farm workers harvest 7–10 tons of sugarcane through extreme physical effort. To perform sugarcane cutting, a farm worker typically performs approximately 4000 trunk movements and 4000 arm movements during a single day. The effects of these extreme conditions on the health of farm workers are poorly understood.

Mucociliary clearance occurs from the nose to the lower airways and is a critical innate respiratory defense mechanism that removes particles and microorganisms from the respiratory system (Lioté et al., 1989; Nakagawa et al., 2000; Randell and Boucher, 2006). Noxious agents are trapped in mucus that is transported by ciliary activity towards the oropharynx, where it is swallowed or expectorated (Puchelle et al., 1987; Nakagawa et al., 2000; Goto et al., 2010). The efficiency of mucociliary clearance depends on three major components: the volume and composition of the airway surface liquid (mucus and periciliary fluid), the ciliary beat frequency, and the mucociliary interaction. An imbalance in one or more components of this defense mechanism leads to impaired mucociliary clearance, which has been associated with increased susceptibility to respiratory infection (Konrad et al., 1994). The nose is the first line of defense of the respiratory system, and nasal mucociliary clearance can be assessed using the saccharine transit test (Puchelle et al., 1987; Nakagawa et al., 2005; Goto et al., 2010), a test that correlates well with radioisotopic technique to measure mucociliary clearance (Puchelle et al., 1981). The aim of this study is to evaluate nasal mucociliary clearance and the mucus properties of farm workers exposed to biomass-burning emissions during sugarcane harvesting.

2. Materials and methods

2.1. Study population

We consecutively selected 30 male farm workers from an employer's roll of 110 potentially elegible subjects. These subjects consisted of both smokers and non-smokers, aged 21–45 years, who were employed in the agricultural practice of sugarcane culture and lived in Cerquilho City in Brazil. Cerquilho City is a small town of approximately 40,000 inhabitants, surrounded by sugarcane plantations, that is approximately 93 miles from São Paulo City, in São Paulo State. Exclusion criteria included previous nasal surgery, use of medications for chronic illness in the previous three months, acute respiratory disease in the last three months, and the inability to taste saccharine. The Ethical Committee of Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil, approved the protocol. All participants provided informed written consent.

2.2. Protocol

Farm workers were evaluated at the end of two successive time periods: first at the end of a 6-month harvesting period (harvesting) and then at the end of a 3-month period without harvesting (non-harvesting). Although some individuals come from distant areas of Brazil to work in São Paulo State during the harvesting season, the majority of subjects enrolled in this study lived in the Cerquilho City area during both the harvesting and the non-harvesting seasons (with no changes in their place of residence and no other local alternative industrial employment opportunities, such as mining or metal smelting). During the non-harvesting period, from December to February, the subjects worked with sugarcane, were involved in other plantation activities (cleaning and cultivating the land) or were unemployed.

Data collection for both time periods was performed after a 5-day work week at the Heart Institute of Hospital das Clínicas da Faculdade de Medicina, São Paulo State, Brazil. Before being assessed, all farm workers rested for 30 min in a seated position in a quiet room with constant ambient temperature (21–23 °C) and relative humidity (60%). Heart rate (bpm), arterial blood pressure (mmHg), respiratory rate (rpm), body temperature (°C), pulse oximetry (%) and exhaled carbon monoxide (ppm), that was recorded on a Micro Medical Instruments device (Rochester, England).

2.3. Nasal mucociliary clearance

We evaluated nasal mucociliary clearance in all subjects at each of the designated time points using the saccharine transit test. While the patient was seated, a 5 mg particle of saccharine was placed 2 cm inside an unobstructed nostril on the inferior turbinate under visual guidance. A timer was displayed to measure transit time. The saccharine transit test records the elapsed time from the placement of the particle until the subject reports the sweet taste of saccharine (Puchelle et al., 1981; Stanley et al., 1986; Nakagawa et al., 2005; Goto et al., 2010). Subjects were allowed to swallow freely and were asked to maintain normal ventilation, avoiding deep breaths, talking, sniffing, sneezing, eating, or coughing. The normal mean value reported for this assay is 11–12 min in healthy adults (Stanley et al., 1986; Nakagawa et al., 2005; Goto et al., 2010).

2.4. Nasal mucus collection and analysis

Immediately after the saccharine transit test measurement, a sample of nasal mucus was collected from each subject using a soft brush gently placed in the nostril that was not used for the saccharine transit test (Goto et al., 2010). The mucus sample was removed from the brush with the aid of a needle and stored at −70 °C for further *in vitro* analysis of mucus properties. Mucus samples were coded and mucus properties were analyzed by means of contact angle and transportability by sneeze by two investigators who were blinded to the two conditions (harvesting and non-harvesting).

2.5. In vitro mucus analysis

2.5.1. Mucus contact angle

The contact angle is a measure of wettability, which is the tendency of a biological fluid to spread when deposited on a solid plane surface as a result of the interaction between the surface and the fluid molecules. To determine the contact angle using an optical microscope (25x) with a goniometer (scale of 0–180°), we placed each mucus sample on a pretreated plate with sulfochromic acid and adequate ambient humidification (37 °C and 100% relative humidity) (Nakagawa et al., 2004; Daviskas et al., 2009).

2.5.2. Mucus transportability by sneeze

We evaluated mucus transportability by a high airflow with the aid of a simulated cough machine that mimics coughing or sneezing. The machine system consists of a cylinder of compressed air connected to a cylindrical acrylic tube (4 mm internal diameter and 133 mm length) that releases compressed ambient air with an airflow of 235 l/min. Displacement of the mucus sample after a single maneuver is presented in mm (Nakagawa et al., 2005; Redding et al., 2008; Goto et al., 2010).

2.6. Lung function test

Pulmonary function testing was performed using a volume displacement spirometer (Koko model, PDS Instrumentation, Inc., Colorado, EUA) and testing techniques adhering to the recommendations of American Thoracic Society Standartization of Spirometry (1987). The following parameters were recorded: percentage of predicted values for forced expiratory volume in the first second (FEV_1), forced vital capacity (FVC), forced expiratory flow between 25% and 75% of FVC ($FEF_{25-75\%}$) and absolute values of FEV_1/FVC ratio. The predicted values were obtained from equations developed by Pereira et al. (2007).

2.7. Atmospheric sampling at the farm and urban areas

Atmospheric samples were collected in Cerquilho City (urban area) and in the farm area during 6 h per day on three consecutive days after the harvesting and non-harvesting time periods. Standard samples of particulate matter (PM) with an aerodynamic diameter of 2.5 μm ($PM_{2.5}$) were measured with a mass spectrophotometer (Dust Trak^{TM} 8520, TSI Inc., MN, USA). This technique calculates the scatter of infrared light positioned 180° from the sensor. The total light reaching the detector is proportional to the PM concentration. Calibration of the equipment was first performed at TSI Inc., MN, USA, and the standard external calibration performed with the aid of MPS Scanning Mobility Particle Size (TSI Inc., MN, USA).