Effects of Cigarette Smoking Intensity on the Mucociliary Clearance of Active Smokers

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Key Words

Mucociliary clearance · Respiratory epithelium · Smoke inhalation injury · Smoking · Tobacco

Abstract

Background: Smoking impairs mucociliary clearance and increases respiratory infection frequency and severity in subjects with and without smoking-related chronic lung diseases. **Objective:** This study evaluated the effects of smoking intensity on mucociliary clearance in active smokers. **Methods:** Seventy-five active smokers were grouped into light (1–10 cigarettes/day; $n = 14$), moderate (11–20 cigarettes/day; $n = 34$) and heavy smokers (≥ 21 cigarettes/day; $n = 27$) before starting a smoking cessation programme. Smoking behaviour, nicotine dependence, pulmonary function, carbon monoxide in exhaled air (exCO), carboxyhaemoglobin (COHb) and mucociliary clearance measured by the saccharin transit time (STT) test were all evaluated. An age-matched non-smoker group ($n = 24$) was assessed using the same tests. **Results:** Moderate (49 ± 7 years) and heavy smokers (46 ± 8 years) had higher STT ($p = 0.0001$), exCO ($p < 0.0001$) and COHb ($p < 0.0001$) levels compared

with light smokers (51 ± 15 years) and non-smokers (50 ± 11 years). A positive correlation was observed between STT and exCO ($r = 0.4$; $p < 0.0001$), STT and cigarettes/day ($r = 0.3$, $p = 0.02$) and exCO and cigarettes/day ($r = 0.3$, $p < 0.01$).

Conclusion: Smoking impairs mucociliary clearance and is associated with cigarette smoking intensity.

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Introduction

Tobacco smoke exposure is a significant risk factor for developing respiratory diseases, such as lung cancer and chronic obstructive pulmonary disease (COPD) [1]. Despite tobacco use being the leading cause of preventable deaths in the world, approximately 6 million people die from smoking-related diseases, and the World Health Organisation estimates that this number will increase to 8 million in 2030 [2].

Previous studies have indicated that smoking intensity influences the risk of developing lung disease [3–9]. Heavy smokers have a higher relative risk of developing lung cancer and COPD compared with light and moder-

ate smokers, respectively [10, 11]. Cigarette smoking has also been reported to increase the frequency and severity of respiratory infections in subjects with and without smoking-related chronic lung diseases [12].

The most effective strategy to reduce morbidity by cigarette smoking is to reduce the number of current smokers or at least their smoking intensity [13–15].

The complex mixture of thousands of chemical compounds in cigarette smoke is highly toxic [16]. Carbon monoxide (CO) is the major toxic gas present in cigarette smoke. Endogenous CO production is a process linked to oxidative stress, and haeme oxygenase (HO) responds to conditions under which oxidative stress is increased [17, 18]. High levels of oxidants and reactive oxygen species have been detected in cigarette smoke from the mainstream and sidestream inhaled by active and passive smokers, which both deliver a large load of oxidants upon the airway epithelium [19].

The defence mechanism of the respiratory tract is affected by substances present in cigarette smoke [20]. Its proper functioning depends on ciliary beat frequency (CBF), the physical properties and transportability of respiratory mucus, and the interaction between the cilia and the mucus layer [21]. Cigarette smoke causes changes in CBF, and in vitro studies have shown that cotinine, a metabolite of smoking, reduces CBF, and the particulate phase of cigarette smoke reduces the CBF response after a stimulus [22, 23].

Despite evidence that cigarette smoke negatively influences mucociliary clearance and decreases the protective host responses to infection, the effects of cigarette consumption intensity on the impairment in mucociliary clearance have not been fully investigated in smokers. We hypothesise that mucociliary clearance is impaired in smokers with higher intensities of exposure.

Methods

Subjects

A total of 117 participants were enrolled in the present study and notified in advance about its objectives and procedures, and written informed consents were obtained in accordance with the Declaration of Helsinki of the World Medical Association. This study was approved by the Research Ethics Committee (12/2010) of the São Paulo State University, Presidente Prudente, Brazil.

Ninety-three individuals enrolled in this study were smokers before starting the smoking cessation programme conducted in the Outpatient Department of Physical Therapy at the São Paulo State University, Presidente Prudente, Brazil. The inclusion criteria were as follows: smokers of both genders with more than 20 years of smoking. The smokers were classified into light (1–10 cigarettes/day), moderate (11–20 cigarettes/day) and heavy smokers (≥ 21 cigarettes/day) [24]. Smoking intensity was assessed by indi-

vidual self-reporting and confirmed by CO in exhaled air (exCO) values [25]. Individuals with cystic fibrosis, bronchiectasis, immotile cilia syndrome, a history of nasal surgery or trauma, upper airway inflammatory disease, COPD or a tobacco-related disease certified clinically and/or by spirometry were excluded.

After performing the pulmonary function test, 18 smokers were excluded due to the presentation of airway obstruction. Seventy-five smokers remained in the study and were divided into groups of light ($n = 14$), moderate ($n = 34$) and heavy smokers ($n = 27$). Twenty-four age-matched non-smoking healthy controls with normal pulmonary function were also assessed (fig. 1).

Study Design and Protocol

Individuals included in this study were evaluated at the Physical Therapy and Rehabilitation Outpatient Clinic at the São Paulo State University by a first interview conducted to obtain personal data, smoking history (years of smoking, cigarettes/day and pack-year index), nicotine dependence according to the Fagerström questionnaire and spirometric pulmonary function [25, 26]. Individuals were instructed to remain abstinent during the 12 h preceding the tests on the next day. They were also instructed to not use pharmacological agents, such as anaesthetics, analgesics, barbiturates, tranquilisers and antidepressants, or alcohol- and caffeine-based substances during the 12 h preceding the test. exCO, carboxyhaemoglobin (COHb) and saccharin transit time (STT) tests were performed in the morning (from 8 to 9 a.m.); the temperature was set at 24°C, and the relative humidity ranged from 50 to 60% to avoid variation in the parameters analysed.

Pulmonary Function

Spirometry was performed according to the guidelines of the American Thoracic Society using a portable spirometer (version 3.6; Spirobank-MIR, Rome, Italy) [26]. Reference values were those specific for the Brazilian population [27].

Exhaled Carbon Monoxide and Carboxyhaemoglobin

The exCO and COHb parameters were measured using a CO analyser (Micro CO Meter, Cardinal Health, Chatham, UK) [28]. The subjects were instructed to hold their breath for 20 s and then to exhale slowly through a mouthpiece. Two successive recordings were made, and the highest value was used [29].

Saccharin Transit Time Test

Nasal mucociliary clearance was assessed using the STT test previously described by Salah et al. [30]. During this test, each subject was seated with his/her head extended at 10°, and granulated sodium saccharin (5 µg) was then deposited under visual control at approximately 2 cm inside of the right nostril. Nasal mucociliary clearance was measured as the time required for subjects to perceive a sweet taste. If no response was reported after 60 min, the test was concluded after confirming the subject had normal sweet taste perception by placing saccharin powder directly onto the tongue. Individuals were instructed to not breathe deeply, talk, cough, sneeze or sniff during the test.

Statistical Analysis

Data were analysed using GraphPad Prism, 3.0 (GraphPad Software Inc., San Diego, Calif., USA). Normal distribution of data was assessed using the Kolmogorov-Smirnov test. Comparisons

Fig. 1. Study flow diagram.

between groups were conducted by analysis of variance followed by the Tukey method for parametric data (age, spirometric values and years of smoking) or the Kruskal-Wallis test, followed by Dunn's method for nonparametric data (body mass index, Fagerström questionnaire, exCO, COHb, pack-year index, cigarettes/day and STT). Data are presented as mean (SD) and median values (ranges: 25th and 75th percentiles) according to normality. Linear correlations were evaluated using Spearman's correlation coefficient. Statistical significance was defined as $p < 0.05$.

Results

This study was conducted between July 1, 2010, and July 15, 2011, and included 75 smokers divided according to cigarette consumption into groups of light ($n = 14$), moderate ($n = 34$) and heavy smokers ($n = 27$), and 24 age-matched non-smoking individuals. There was no significant difference in demographic characteristics (gender, age and body mass index) and spirometric values between groups (table 1).

The years of smoking parameter (mean $\pm$ SD) was similar ($p = 0.23$) between light (34 ± 13 years), moderate (30 ± 8 years) and heavy smokers (34 ± 5 years). Heavy smokers had a higher pack-years index compared with moderate and light smokers ($p < 0.0001$). The daily consumption of cigarettes was higher in heavy smokers compared with moderate and light smokers ($p < 0.0001$), while moderate smokers showed higher cigarette consumption per day than light smokers ($p < 0.0001$; table 2).

There was no significant difference in nicotine dependence between groups according to the Fagerström questionnaire (table 2).

Moderate and heavy smokers had higher STT results than light smokers and non-smokers (fig. 2a; $p = 0.0001$) and also showed higher exCO (fig. 2b; $p < 0.0001$) and COHb (fig. 2c; $p < 0.0001$) values. A positive correlation was observed between STT and exCO (fig. 3a; $r = 0.4$; $p < 0.0001$); STT and cigarettes/day (fig. 3b; $r = 0.3$, $p = 0.02$), and exCO and cigarettes/day (fig. 3c; $r = 0.3$, $p < 0.01$).

Table 1. Demographic characteristics and spirometric data of the subjects according to the cigarette smoking intensity

Characteristics	Smokers			
	non (n = 24)	light (n = 14)	moderate (n = 34)	heavy (n = 27)
<i>Demographics</i>				
Males, %	30	38	41	48
Age, years	50±11 47 (40–60)	51±15 55 (42–64)	49±7 50 (44–55)	46±8 48 (41–52)
BMI	26±4 24 (23–30)	25±3 24 (22–27)	26±4 27 (23–29)	26±5 26 (24–28)
<i>Spirometric values</i>				
FEV ₁ , % of predicted	97±13 96 (85–104)	101±102 101 (89–112)	93±11 92 (85–105)	94±14 95 (82–109)
FVC, % of predicted	93±11 93 (88–96)	97±11 96 (87–107)	96±11 96 (87–106)	102±15 103 (91–116)
FEV ₁ /FVC, %	82±2 82 (80–83)	87±2 87 (84–90)	81±6 82 (78–85)	82±6 81 (77–83)

Data are presented as means ± SD and medians (ranges). BMI = Body mass index; FEV₁ = forced expiratory volume in 1 s; FVC = forced vital capacity.

Discussion

This study showed a higher impairment in mucociliary clearance and higher levels of exCO and COHb in moderate and heavy smokers compared with non-smokers and light smokers with similar years of smoking.

Previous studies have established a correlation between the nasal and tracheobronchial mucociliary clearance rate, which highlights the advantage of the nasal STT test as a simple, low-cost, less-invasive method to assess mucociliary function [31, 32]. Furthermore, nasal mucociliary clearance measured with the STT test correlated well with CBF and is widely used in recent studies [33–36].

The efficacy of mucociliary clearance depends on (a) the quality and quantity of the mucus and the depth of the periciliary layer; (b) the synchrony of CBF and structure of the respiratory epithelium, and (c) the interaction between the cilia and mucus [37, 38]. The impairment in mucociliary clearance in individuals chronically exposed to cigarette smoke is associated with epithelial remodeling leading to structural abnormalities in the cilia, goblet cell metaplasia and mucous cell hypertrophy, resulting in increased mucus production [38, 39].

Beyond the impairment in the mucosal surface, tobacco smoke also affects circulating immune cells and local innate immune cell functions (macrophages and neutro-

Table 2. Tobacco smoking behaviour of subjects according to the cigarette smoking intensity

Characteristics	Smokers		
	light (n = 14)	moderate (n = 34)	heavy (n = 27)
<i>Smoking behaviour</i>			
Years of smoking	32±17 31 [18–48]	29±9 30 [23–35]	32±8 33 [27–38]
Pack-year index	17±10 18 [10–23]	27±11 24 [19–35]	59±44 ^a 50 [40–68]
Cigarettes/day	9±1 10 [9–10]	18±3 ^b 20 [15–20]	39±11 ^a 40 [30–41]
Fagerström questionnaire score	4±2 3 [3–6]	5±2 5 [3–6]	6±3 6 [3–7]

Data are presented as means ± SD and medians (ranges). ^a p < 0.0001 vs. moderate and light smokers, ^b p < 0.0001 vs. light smokers.

phils) [40]. These changes, which are associated with airway remodelling and reduced respiratory function, increase the risk for developing COPD and infection by tuberculosis, bacterial pneumonia and influenza [41–43].

In the present study, moderate and heavy smokers had a similar impairment in mucociliary clearance, and light

Fig. 2. STT (a), exCO (b) and COHb (c) of non-smokers (n = 24), and light (n = 14), moderate (n = 34) and heavy smokers (n = 27). Data are presented as medians and ranges. * $p \leq 0.0001$ vs. non-smokers and light smokers.

Fig. 3. A positive correlation was observed between the values of the STT and exCO (a; $r = 0.4$; $p < 0.0001$); STT and cigarettes/day (b; $r = 0.3$; $p = 0.02$) and exCO and cigarettes/day (c; $r = 0.3$, $p < 0.01$).

smokers showed mucociliary clearance similar to non-smokers. Previous studies have suggested that the mucus rheology in light smokers could favour mucociliary clearance [44, 45], and an increased baseline CBF in the nasal epithelium of heavy smokers, similar to light smokers, has been demonstrated compared with non-smokers [46]. These reports suggest that the differences in mucociliary clearance found in the present study could be rather related to the mucus and depth of the periciliary layer than to CBF.

Recently, it has been shown that exposure to cigarette smoke reduces CFTR (cystic fibrosis transmembrane conductance regulator) expression, resulting in airway surface liquid depletion and mucus stasis in the airway epithelium [47]. Thus, the mechanism linking reduced mucociliary clearance with smoking intensity could be at least partially due to the inhibition of CFTR-dependent airway fluid transport. Future studies should address this issue.

Moderate and heavy smokers had similar exCO and COHb values, whereas light smokers presented values similar to non-smokers. These findings may be explained by previous studies that found high concentrations of free radicals in the cigarette gas phase, which results in increased oxidative stress at higher exposure intensities [48, 49]. Studies have reported that exCO is present in inflammatory lung diseases and reflects the induction of HO-1 and oxidative stress that impaired mucociliary clearance [44, 50]. Thus, the high levels of oxidative stress may ex-

plain the diminished nasal mucociliary clearance in smokers with increased exCO.

Additionally, the correlation between exCO and the level of daily cigarette consumption has been well established. This study also observed a positive correlation between exCO and cigarettes/day [51]. The STT values were positively correlated with cigarettes/day and exCO, which demonstrates that individuals with impaired mucociliary clearance reported a higher daily consumption of cigarettes and higher exCO values, thereby corroborating the previous study [52].

Our results showed that light smokers have evident benefits on mucociliary clearance compared with moderate and heavy smokers. However, our study has some limitations: we did not evaluate airway remodelling or the influence of the reduced mucociliary clearance on the frequency of respiratory infection. Future studies should clarify these issues.

In summary, we have shown that smoke impairs mucociliary clearance, which is associated with cigarette smoking intensity.

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