Short-term smoking reduction is associated with reduction in measures of lower respiratory tract inflammation in heavy smokers


ABSTRACT: The beneficial effect of short-term smoking reduction in reducing lower respiratory tract inflammation was assessed in 15 healthy heavy smokers. All underwent fiberoptic bronchoscopy and bronchoalveolar lavage and were then treated with at least 20 mg of nicotine gum daily. Self-reported cigarette consumption decreased from 50.7±2.3 to 18.8±1.5 (p<0.001) cigarettes daily, and expired CO decreased from 48.5±2.5 to 27.3±2.5 ppm (p<0.001). After two months, repeat bronchoscopy and bronchoalveolar lavage revealed that bronchial inflammation, as assessed by direct inspection, neutrophilia of bronchial lavage fluid, and the number of alveolar macrophages, the number of alveolar neutrophils and the concentration of neutrophil elastase alpha-antiprotease complex in alveolar lavage fluid, had improved significantly. The present study suggests that smoking reduction may be associated with an improvement in lower respiratory tract inflammation in heavy smokers and may, if persistent, be an alternative for smokers who cannot, or do not wish, to quit.


Cigarette smoking continues to be an extremely tenacious addiction. Current evidence indicates that nicotine, like alcohol and opiates, is a potent psychoactive, dependence-producing drug [1, 2]. Most smokers state that they want to quit, but only one in three stops permanently before the age of 60 yrs [3]. While smoking cessation is unquestionably the most important therapeutic goal of intervention, there are many smokers who never achieve permanent abstinence. For them, reducing the degree of lifetime cigarette exposure may be a therapeutic alternative. In support of this, there is cross-sectional data demonstrating a relationship between dose of smoking and smoke-related bronchial carcinoma [4]. This makes it reasonable to explore possible health benefits of reducing smoking in a defined population.

The present study was designed to assess whether smoking reduction would be associated with evaluable benefits that might be a reasonable therapeutic goal. We specifically sought to determine whether smoking reduction in a group of heavy smokers would be associated with measurable improvement in abnormalities which are present in the lower respiratory tract of smokers, including increased macrophages, increased neutrophils, and increased levels of the neutrophil derived protease, elastase. Reduction was achieved in the study group, and it was associated with improvements in lower respiratory tract inflammation. These results suggest that smoking reduction may be a reasonable therapeutic goal in heavy smokers who cannot or do not wish to quit.

Methods

Patients

Fifteen healthy volunteers (aged 21–44 yrs), who consumed a minimum of two packs of cigarettes daily, were studied under a University of Nebraska Medical Center Institutional Review Board approved protocol, after informed consent had been obtained. Heavy smokers were chosen for two reasons. Firstly, as observed by JAFFE and KANZLER [2], they tend to be the most sensitive to elevated plasma nicotine levels. Such smokers reduce their cigarette intake when the plasma nicotine levels become unusually high. This made it seem more likely that the smokers could achieve reduction when exogenous nicotine was administered (see below). Secondly, it was expected that the measurable effects of
smoking would be easier to observe in heavy smokers. Because this was to be a smoking reduction study, all volunteers who expressed a current interest in quitting were encouraged to do so and were excluded from the investigation. All participants (9 males, 6 females) had normal results on physical examination, ECG, chest radiograph, and blood chemistries. Spirometry, except for reduced flows at low lung volumes, was also normal. All subjects completed all phases of the study.

For comparison purposes, a group of 15 normal, nonsmoking volunteers (aged 18-36 yrs) were also studied. All had normal physical examinations, pulmonary function tests, chest radiographs and no history of any lung disease. All underwent bronchoscopy and bronchoalveolar lavage under a University of Nebraska Institutional Review Board approved protocol after informed consent had been obtained. One subject who had obvious evidence of bronchitis at the time of bronchoscopy was questioned again after the study and admitted to smoking marijuana cigarettes. This subject was an "outlier" for several of the parameters assessed. As "exit interviews" were not routine and as the subject met all "entry" criteria, it was felt to be most valid to include this subject in the normal group for statistical purposes. Exclusion of this subject only increases the differences observed.

**Procedures**

**Smoking reduction.** All smokers agreed to reduce their cigarette consumption by 50%. Several lines of evidence suggested that nicotine replacement therapy may be a reasonable aid to achieve smoking reduction. Firstly, smokers tend to adjust their nicotine intake to their individually preferred levels [5, 6]. Secondly, smokers reduce their smoking when nicotine is administered by aerosol or intravenously [7, 8]. Thirdly, smokers significantly reduce their cigarette consumption following treatment with gum delivering 4 or 8 mg nicotine [8]. Therefore, all subjects in the current investigation were instructed to use at least ten 2 mg nicotine-polariflex gum (Nicorette, Merrell Dow) daily. By administering nicotine through the gum, it was hoped that desire to smoke would decrease making reduction easier to achieve. The effectiveness of this intervention as a strategy for smoking reduction was not compared with other interventions, although the smoking behaviour and the subjective responses of the subjects were recorded. Participants were given forms on which to record daily cigarette and nicotine gum consumption. To confirm smoking history, expired carbon monoxide (CO) was measured (Interscan Model No. 1146, Interscan Corp., Chatsworth, CA) at entry and at one and two months [9]. The time of day of the testing was controlled by scheduling the two month CO assessment at roughly the same time as the entry meeting. Expired CO was also measured at one month, but the time of testing was arranged for patient convenience, generally late afternoon or early evening.

**Bronchoscopy**

To determine whether a reduction in cigarette consumption would result in reduced lower respiratory tract inflammation, participants underwent a bronchoscopy and bronchoalveolar lavage (BAL) [10, 11] prior to starting their smoking reduction programme. In all cases, smokers were given topical anaesthesia to permit the flexible fibreoptic bronchoscope to be passed transorally. This procedure was repeated at the conclusion of the two month smoking reduction trial. For baseline comparisons only, 15 normal nonsmoking volunteers were evaluated once.

**Bronchitis index**

During bronchoscopy, two physicians independently rated bronchial inflammation by visual examination. Four characteristics, friability, erythema, oedema and secretions were rated on a four point scale; 0-3 in each of the five lobes and lingula [11]. Normal = a score of 0; mild = 1; moderate = 2; severe = 3. The sum of each of the four rated characteristics was totalled from all six lung sites, and the two independent observations were averaged to develop a final bronchitis index. The correlation between the two scorers was excellent, r=0.92, p<0.001.

**Bronchoalveolar lavage**

Bronchoalveolar lavage was performed after gently wedging the bronchoscope in a segmental or subsegmental bronchus [10, 11]. Five 20 ml aliquots of sterile saline were infused into each of three lobes. The fluid from the first aliquot in each lobe, enriched for bronchial material, was collected and analysed separately [11, 12]. The remaining four aliquots from each lobe, which were enriched for alveolar material, were pooled.

**Cytological examination and elastase determination**

To prepare samples for cytological examination, BAL fluid was filtered through a nylon gauze to remove excess mucus. Total cell numbers were counted prior to further processing using a haematocytometer. One hundred thousand cells were centrifuged at 165 g on a cytocentrifuge (Shandon) for 10 min and stained using Diff-Quik (American Scientific). Cell differentials were counted twice on 200 cells and averaged in both the bronchial and alveolar samples. Blood and lavage samples were stored for later analysis of urea and albumin concentrations [13, 14] at -80°C. To evaluate elastase burden, elastase activity was assayed using a synthetic substrate [15] and elastase alpha,-antitrypsin complex in the alveolar samples was assayed with a sandwich method enzyme-linked immunosorbent assay (ELISA) [16]. Both urea, measured enzymatically, and albumin,
measured by ELISA, were determined and used to
correct for variable dilution of the lung epithelial lining
fluid by the saline during the BAL [13].

Statistics

Comparisons between smokers and normals were made
with the unpaired Wilcoxon test. Student's paired T-test
was utilized to assess the two month changes in CO
values and average cigarette consumption. The paired
Wilcoxon test was used to assess changes in all BAL
parameters.

Results

Smoking reduction assessment

All participants reported a consistent decrease in
cigarette consumption (fig. IA). Overall, self-reported
cigarette consumption fell from 50.7±2.3 at entry to
18.8±1.5 cigarettes a day after two months (p<0.001).
Reduction was confirmed by expired CO which decreased
from 48.5±2.5 ppm to 27.3±2.5 ppm at two months
(p<0.001) (fig. IB). The mean expiratory CO
concentration at one month seems lower than at two
months, suggesting some increase in smoking during the
second month. As the measurements at the beginning
and end were controlled for time of day and that at
one month was not, however, this inference is not
definitive.

Fig. 1. - Smoking status. A: Smoking status as
determined by self-report. Vertical axis: cigarettes per day; horizontal axis: weeks of cessation.
B: Smoking status as assessed by expired CO. Vertical axis: expired CO (ppm); horizontal axis: weeks of study.

Even though smokers achieved smoking reduction,
all but two reported that the task was difficult. Fourteen
participants reported symptoms which may have been
secondary to nicotine withdrawal: craving for cigarettes
(n=14), irritability (n=12), anxiety (n=10), restlessness
(n=10), difficulty concentrating (n=8), headache (n=6),
and drowsiness (n=3).

Bronchitis index

Despite the fact that all study subjects were selected to
be asymptomatic and have normal pulmonary functions,
abnormally appearing airways consistent with mild
bronchitis was regularly observed (fig. 2). The mean
bronchitis index of smokers was 8.5±0.9, significantly
increased compared to the normal nonsmokers, 1.9±0.8
(p<0.01). Smoking reduction was associated with a
significant reduction in the visible evidence for
bronchitis as the mean bronchitis index fell to 6.5±0.6
(p<0.05).

Cell recovery by bronchoalveolar lavage

As expected, increased numbers of cells were
recovered from the BAL of smokers. This was entirely
due to an increased recovery of cells in the alveolar
sample (fig. 3) and not to an increase in cells recovered
in the bronchial sample which were no different among
the three study groups. The mean recovery of cells in
the alveolar lavage of smokers prior to reduction was
104.3±9.4 × 10^6, approximately fourfold greater than that.
of normal nonsmoking volunteers, 27.3±3.13 x 10⁶ (p<0.001). After smoking reduction, mean alveolar cell recovery was 73.1±10.3 x 10⁶, representing a significant decrease compared to values before reduction (p<0.01), but still increased compared to normal nonsmoking volunteers.

The increased recovery of cells in the alveolar samples of normal smokers, and the decrease following smoking reduction was not due to variable recovery of lavage fluid. When expressed per ml of lavage fluid recovered, normal smokers had increased mean concentration of cells (0.69±0.07 x 10⁶ cells·ml⁻¹) recovered in lavage fluid compared to normal nonsmokers (0.16±0.02 x 10⁶ cells·ml⁻¹), and this decreased significantly after smoking reduction (0.52±0.06 x 10⁶ cells·ml⁻¹) (p<0.01). The same relationship was also observed when the absolute concentrations of cells in lung epithelial lining fluid were estimated using urea as an internal marker of dilution: 52.1±10.4 x 10⁶ cells·ml⁻¹ before vs 27.4±2.0 x 10⁶ cells·ml⁻¹ after reduction compared to 10.4±1.1 x 10⁶ cells·ml⁻¹ in normal nonsmokers (p<0.05, all comparisons). There was no difference in the mean number of cells recovered in the bronchial samples among the three groups, (4.1±0.6 x 10⁶ normals, 3.6±0.6 x 10⁶ smokers before and 5.9±1.3 x 10⁶ smokers after reduction). Bronchial sample cell recoveries were also similar when compared on a concentration basis using either volume of recovered lavage or urea in the bronchial sample (data not shown).

Analysis of cell types recovered by bronchoalveolar lavage

Macrophages comprised the majority of cells recovered in both the bronchial and alveolar samples. As expected, bronchial samples were relatively enriched for ciliated epithelial cells compared to the alveolar samples for both study groups; they were also enriched for neutrophils. Smokers had a significantly greater mean percentage of neutrophils (20.7±2.6) in their bronchial samples than did nonsmokers (9.6±1.2, p<0.01) and this decreased with smoking reduction to 12.6±2.0 (p<0.05, fig. 4A). Smokers also had a significantly greater percentage of neutrophils in their alveolar samples (5.7±1.3) which also tended to decrease with smoking reduction (3.3±0.8) (fig. 4B), but this did not reach statistical significance (p=0.06). The total number of neutrophils in the alveolar sample of smokers, however, decreased significantly with reduced cigarette intake (5.9±1.5 x 10⁶ before vs 2.6±0.7 x 10⁶ after, p<0.05) (fig. 5).

Neutrophil elastase

Since one mechanism by which neutrophils can injure the lung is thought to be through the release of the potent protease, neutrophil elastase, neutrophil elastase activity was assayed in BAL fluids. No fluid contained active elastase. Using an ELISA method, however, antigenic
neutrophil elastase; alpha,-antitrypsin complex could be detected in the alveolar lavage fluids of the normal smokers. The amount of antigenic elastase complex (adjusted for albumin) was significantly elevated in smokers compared to nonsmokers and decreased significantly with smoking reduction (p<0.01) (fig. 6). Estimating elastase complex concentration in lung epithelial fluid using urea yielded similar findings. The lack of activity of the neutrophil elastase could be accounted for, since all of the detectable neutrophil elastase was present as complex with alpha,-antiprotease.
Discussion

The present study suggests that smoking reduction may be a potential therapeutic goal for heavy smokers who cannot or who do not wish to quit. Short-term smoking reduction was clearly associated with an improvement in a number of inflammatory features present in the lower respiratory tract, specifically: a decrease in the severity of visually observed, but subclinical, bronchitis; a reduction in the number of neutrophils present in the lower airways; and a decrease in the amount of neutrophil elastase detected in the lung. The potential benefits of long-term smoking reduction remain to be determined, but may be reasonable to explore as a therapeutic option in selected smokers.

The subjects of the current study were all "normal" smokers in that clinical assessment, chest X-ray and spirometry, except for reduced expiratory flows at low volumes, were all normal. Biopsies would undoubtedly provide the best measure of lower respiratory tract inflammation. Nevertheless, while biopsies were not performed, several lines of evidence suggest improvement in lower respiratory tract inflammation: 1) reduction in inflammatory cells; 2) reduction in an inflammatory cell mediator, neutrophil elastase; and 3) improvement in directly observed airways inflammation.

In an autopsy study of normal smokers, Niemojner et al. [17] demonstrated a consistent accumulation of pigment-laden macrophages in the respiratory bronchioles, a lesion termed "small airways disease" [17, 18]. It has been suggested that "small airways disease" may be the precursor of fixed obstructive lung disease [19-23]. Thus, improvement in "small airways disease" might be an initial goal in the prevention of emphysema. The BAL correlate of "small airways disease" is unknown. Smokers, however, consistently have increased recoveries of pigment-laden macrophages compared to nonsmokers [24, 25]. It is likely that the macrophages which accumulate in the airways of the respiratory bronchioles, the hallmark basis of small airways disease, are being sampled by BAL. The findings in the present study, that the number of alveolar macrophages recovered decreases with smoking reduction is, therefore, consistent, with an improvement in the small airways inflammation following smoking reduction.

The mechanisms which lead to fixed airways obstruction are not completely understood. Cigarette smoke, however, can lead to the release of neutrophil chemotacticants [25], and the elastase contained in the secretory granules of these cells can degrade the structural proteins of the lung connective tissue, thus contributing to lung destruction [26, 27]. In addition, elastase infusion into airways can cause the goblet cell metaplasia characteristic of chronic bronchitis [28]. The current study confirms earlier observations that smokers have increased numbers of alveolar neutrophils recovered by bronchoalveolar lavage [24-26]. It also confirms the detection of neutrophil elastase [29, 30] in the lower respiratory tract. While all of the elastase present appeared to have been inactivated by alpha-antiprotease, in contrast to the previous study of Janoff et al. [29], variations in method of elastase detection and in the protocol of smoking prior to bronchoscopy probably account for the differences. Nevertheless, it is likely that prior to its inactivation, the elastase has the potential to attack lung structural proteins. The findings in the present study, that both total alveolar neutrophils and neutrophil elastase decrease following smoking reduction, suggest that the "elastase burden" of the lower respiratory tract can be reduced by smoking reduction. Clearly, not all smokers develop emphysema, and the determinants of individual susceptibility are not fully known. Thus, while the connection between elastase burden and emphysema is not fully understood, it is likely that a reduction in elastase burden may lessen the risk of developing emphysema.

Chronic bronchitis is a frequent consequence of chronic cigarette smoke exposure. While none of our subjects had the clinical criteria for a diagnosis of chronic bronchitis, all were heavy smokers and had visible abnormalities of the lower respiratory tract. Since our subjects were paid volunteers, it is possible that they had bronchitis, but denied symptoms. Evaluation of patients with the clinical diagnosis of chronic bronchitis, however, has revealed more severe abnormalities for both the visually observed airways abnormalities and for the associated bronchial neutrophilia than those seen in the current study population [31, 32]. Thus, it seems likely that the visually observed bronchitis and bronchial neutrophilia represents a subclinical abnormality. Whether it represents an early lesion which would progress to clinically manifest disease is unclear. Whether smoking reduction would have a similar effect in patients with clinically manifest bronchitis is, therefore, unknown.

Smoking reduction in the current study, however, was not easily achieved, a very high number of symptoms associated with nicotine withdrawal being reported. A different strategy for reduction may have been better tolerated. There was a similar decrement in expired CO than in the self-reported cigarette consumption in our study group. Smokers who reduce smoking compensate for decreased numbers of cigarettes by smoking each more deeply and thoroughly [5]. This suggests that the nicotine replacement used was not completely effective.

Nicotine is a biologically active drug [34, 35]. It may be directly responsible for some of the adverse effects of cigarette smoking and, thus, while nicotine replacement may reduce some of the hazards of cigarette smoking, other strategies to achieve smoking reduction may have much greater benefit than nicotine replacement. Moreover, a direct effect of the nicotine gum as an anti-inflammatory is not excluded by the data available.

Caution must be exercised in interpreting the implications of this study. The data presented are not unequivocal support for smoking reduction as a therapeutic strategy, but merely show improvement in subclinical lower respiratory tract inflammation. It is not known whether similar inflammatory changes and improvements with smoking reduction could be observed in lighter smokers. Smoking reduction will never be a
substitute for cessation. Nevertheless, in heavy smokers who cannot achieve cessation, the present study suggests reduction may be a therapeutic option, in so far as it is definitely sustained. The best strategy to achieve permanent smoking reduction remains to be determined. Based on the results of the current study, a prospective double-blind investigation of the long-term results of smoking reduction techniques seems warranted. Finally, using BAL to assess lower respiratory tract inflammation promises to provide objective criteria by which the efficacy of smoking reduction or cessation can be assessed, even in a clinically normal study population.

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References


RÉSUMÉ: Un effet bénéfique d'une réduction à court terme de l'habitude tabagique, dans les sens d'une diminution de l'inflammation du tractus respiratoire inférieur, a été apprécié chez 15 grands fumeurs sains. Tous ont subi une fibro-bronchoscopie et un lavage broncho-alvéolaire, et furent traités ensuite par au moins 20 mg de chewing-gum à la nicotine par jour. La consommation de cigarettes a diminué, selon leurs déclarations, de 50.7±2.3 à 18.8±1.5 cigarettes par jour (p<0.001). Le CO expired a diminué de 48.5±2.5 à 27.3±2.5 ppm (p<0.001). Après deux mois, une nouvelle fibroscopie avec lavage broncho-alvéolaire a montré que l'inflammation bronchique estimée par l'examen endoscopique, la neutrophilie du liquide de lavage bronchique, et le nombre de macrophages alvéolaires, ainsi que le nombre de neutrophiles alvéolaires et la concentration du complexe élastase neutrophile alpha-antiprotéase dans le liquide de lavage alvéolaire, s'étaient tous améliorés de façon significative. Cette étude suggère que la réduction du tabagisme peut s'accompagner d'une amélioration de l'inflammation du tractus respiratoire inférieur chez les grands fumeurs, et pourrait être, si elle persiste, une alternative pour les fumeurs qui ne peuvent ou ne désirent pas cesser.

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