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Effects of oral doxofylline on inflammatory changes and altered cell proliferation in chronic obstructive bronchitis

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Abstract. – **The effects of oral doxofylline (400 mg BID) on bronchial airway mucosa were investigated in 14 patients with chronic obstructive bronchitis. The eligible patients had to have forced expiratory volume in 1 second > 60% of the predicted value and oxygen partial tension > 55 mm Hg. At the onset and the end of the study, bronchial biopsies were performed via a flexible fiberoptic bronchoscope. Chronic inflammation of the airways was graded according to absence of lesions = 0, involvement of 1-10% = 1, 10-50% = 2 and > 50% = 3. After three months of treatment, 57% of patients in the doxofylline group presented absence of lesions, while the remaining 43% exhibited advanced lesions. In the control group, absence of lesions was observed in 14%, while lesions of grade 1, 2 and 3 were visible in 14%, 29% and 43%, respectively. At histological examination, a significant difference in the degree of structural changes was observed in the doxofylline group between baseline and the end of the study ($p < 0.03$).**

In conclusion, doxofylline may induce favorable effects on inflammatory changes and altered cell proliferation of the airway mucosa in patients with chronic obstructive bronchitis.

Key Words:

Doxofylline, Anti-inflammatory, Bronchodilation, Chronic bronchitis.

Introduction

Doxofylline ([7-(1,3-dioxolan-2-ylmethyl) theophylline] is a methylxanthine bronchodilator lacking of affinity for adenosine receptors with remarkable anti-bronchospastic properties[1,2].

The drug has been shown to possess antitussive activity on experimentally induced citric acid or histamine aerosol cough[3]. Oral doxofylline proved to be useful in reducing symptoms in patients with chronic obstructive bronchitis and productive cough. In association with adequate hydration, mucolytics and humidification, the drug significantly reduced wheezing as well as the intensity and frequency of cough[4].

Since inflammatory stimuli are important mechanisms related to the pathogenesis of wheezing and cough in chronic obstructive bronchitis, it was interesting to investigate whether the favorable effect of doxofylline in these patients may be due to a reduction in chronic inflammation and altered cell proliferation. Therefore, the present study was designed assess the effects of oral doxofylline on respiratory mucosa of patients with chronic obstructive bronchitis at histogical examination of bronchial biopsies.

Patients and Methods

Fourteen patients diagnosed with stable chronic obstructive bronchitis were selected for entering into the study. Patients were considered eligible to participate in this study if they met the following criteria: forced expiratory volume in 1 second (FEV_1) > 60% of the predicted FEV_1 for their age and height, oxygen partial tension > 55 mmHg, smokers for at least 10 years of at least 20 and no more than 40 cigarettes a day and with normal electrocardiogramm, platelet count and time of Quick. The exclusion criteria were: infective exacerbation of chronic obstructive bronchitis, administration of inhaled or systemic corticosteroids or antibiotics.

After the screening visit, the eligible patients were randomly allocated into two study groups: the doxofylline group (n = 7) and the

control group (n = 7). In the treatment group, doxofylline 400 mg BID was given orally for three months in combination with the standard therapy. Standard therapy included beta-2-agonist, anticholinergic agents and mucolytics. Methylxanthine drugs were withheld at least 48 hours before enrolment. Patients in the control group received the same treatment of those of the doxofylline group with the exception of methylxanthines.

Patients were examined at the beginning of the study and after three months. At baseline and at the termination of the study, each patient was submitted to bronchial biopsy performed via a flexible fiberoptic bronchoscope. Within 24 hours from the procedure, the study patients underwent FEV_1 by spirometry and arterial blood samples were drawn for measurements of oxygen and carbon dioxide partial tensions and the pH.

A total of three tissue samples were obtained from each patient during broncoscopy. Histology was evaluated by light microscope in haematoxylin and eosin, Van Gieson, alcian PAS and PAS stained sections. The presence or absence of neutrophilic infiltration, oedema, fibrosis and epithelial metaplasia was assessed in a blind manner in each microscopic field. The final score was based on the percentual involvement of the examined area: absence of lesions = 0, involvement of 1-10% = 1 (mild), 10-50% = 2 (moderate) and > 50% = 3 (advanced).

The two-tailed Student's test for unpaired data was applied to compare values of FEV_1 and blood gas analysis. The Kruskal-Wallis one-way nonparametric analysis of variance was used to compare the difference of the scores between the groups. A p value < 0.05 was considered statistically significant.

Results

The characteristics of the study patients are illustrated in Table I. During the observation period, 29% of the patients in the doxofylline and 43% in the control group noticed deterioration of symptoms. At the end of the trial, a positive judgement on therapeutic efficacy of treatments was expressed by 57% of patients in the doxofylline group and by 43% of the controls.

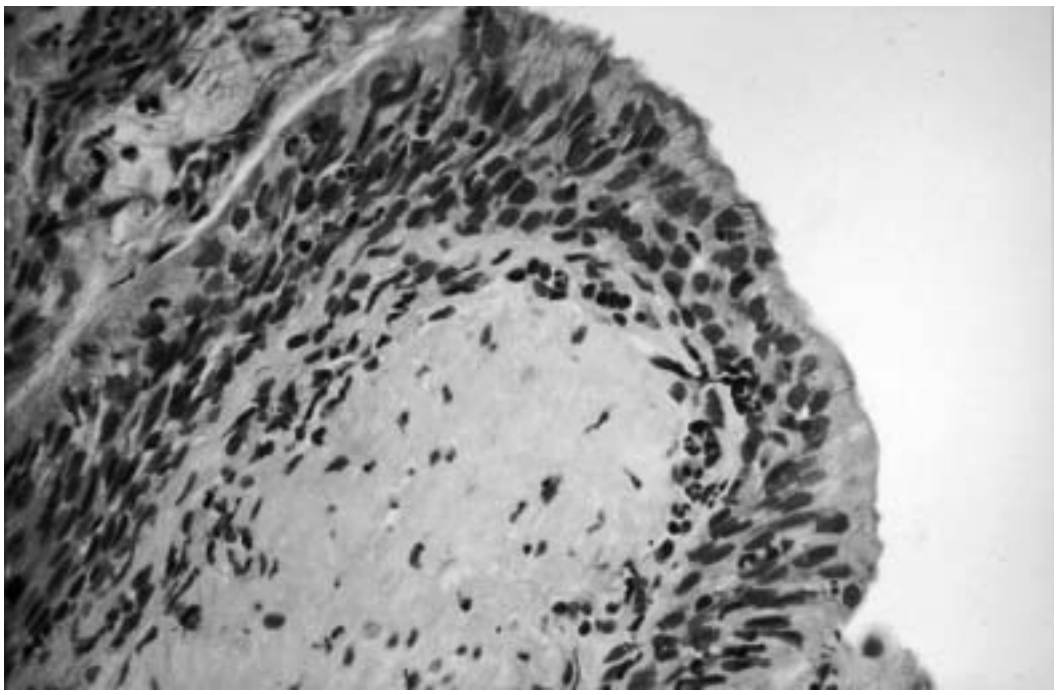
Table I. Characteristics of the study population at baseline (standard deviations in brackets).

	Doxofylline group	*Control group*
Age (years)	61 (9)	60 (8)
Percent of women	14%	29%
Number of exacerbations of the previous year	2 (0.82)	1.86 (0.69)
Number of cigarettes/day	24.2 (5.3)	21.4 (2.4)
Years of smoke	39 (10)	31 (9)
FEV_1 (liters)	2.06 (0.46)	2.2 (0.27)
Oxygen partial tension (mmHg)	74.1 (8.3)	74 (5.7)
Time of Quick (%)	92.8 (7.4)	96.4 (3.6)
Platelet count ($\times$ 103/µl)	264 (45)	287 (37)

FEV_1 did not change significantly at the end of the study with respect to basal values either in the doxofylline group (2.14 ± 0.46 liters versus 2.06 ± 0.50 liters) or in the control group (2.2 ± 0.26 liters versus 2.2 ± 0.27 liters). Oxygen partial tensions slightly increased at the end of the treatment period with respect to baseline in the doxofylline group (75 ± 8.3 mmHg versus 74.1 ± 8.3 mmHg), while slightly decreased in the control group (73.4 ± 5 mmHg versus 74 ± 5.7 mmHg). No changes were reported for both carbon dioxide partial tensions and pH in the two groups.

By light microscopy, histologic lesions such as oedema, interstitial fibrosis and infiltration of inflammatory cells were found at baseline in all patients of the doxofylline group and in 86% of the controls. Grade 1 lesions were observed in 29% of patients in the doxofylline group and in 43% of those in the control group. Grade 2 lesions were found in 14% of both the doxofylline group and controls. Grade 3 lesions were apparent in 57% of the doxofylline group and in 29% of the control group.

After treatment with doxofylline, 57% of patients presented absence of lesions, while the remaining 43% exhibited advanced morphologic lesions (Figure 1). In the control group, absence of lesions after three months of standard therapy was observed in 14%, while lesions of grade 1, 2 and 3 were visible in 14%, 29% and 43%, respectively. Metaplasia of the respiratory epithelium at months 3 was found in two control cases.



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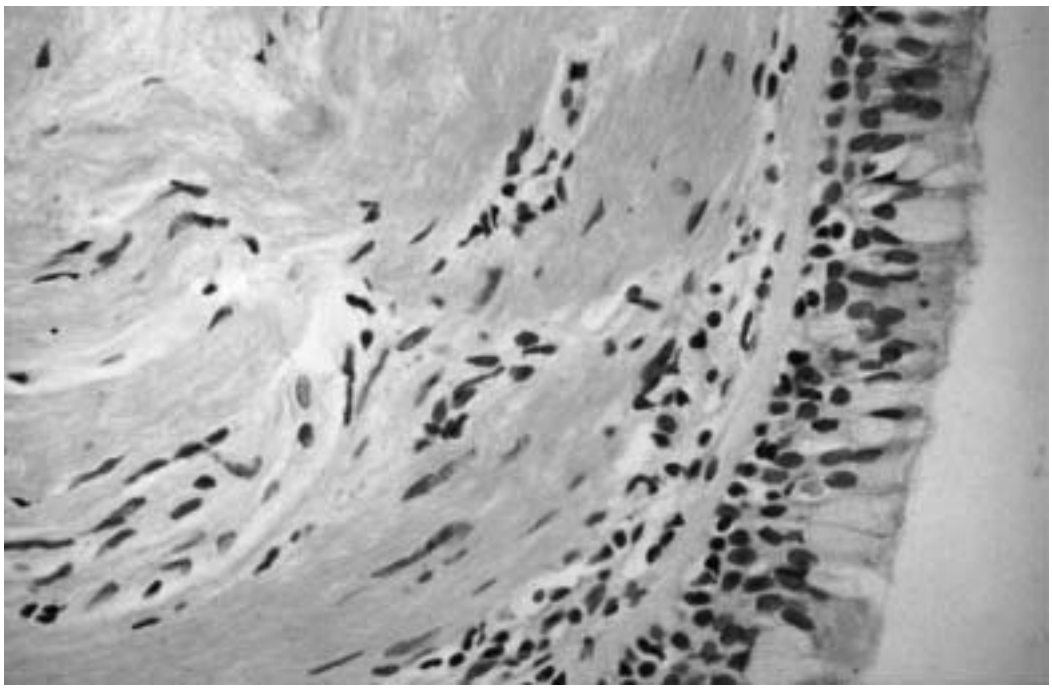

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Figure 1. A. Section showing neutrophilic infiltration of the bronchial wall in a patient with chronic obstructive bronchitis. B. After 3 month treatment with oral doxofylline 400 mg BID, a marked reduction in the histologic abnormalities was apparent.

Comparisons between of the histologic score at baseline at the end of the observation period showed a statistical significant improvement in the group treated with doxofylline ($p < 0.03$), while no significant difference was found in the control group (Figure 2).

Discussion

Since therapeutic benefit of methylxanthines in chronic obstructive bronchitis does not always correlate with the changes in pulmonary function tests, it has been suggested that different mechanisms – like anti-inflammatory activities or preservation of the mucociliary blanket of the airways – may contribute to the usefulness of these drugs in controlling chronic cough and sputum production[5,6].

It is well known that chronic obstructive bronchitis is characterized by inflammatory and obliterative changes of the small airways of the lung. Histological abnormalities include neutrophilic and lymphocyte infiltration and atrophy of columnar epithelium. Structural changes of bronchial airways are frequently accompanied by development of submucosal gland hypertrophy, mucus hypersecretion and impaired mucociliary clearance with frequent disruption of cilia. Finally, advanced pathological alterations of the small bronchi may lead to the development of interstitial fibrosis and squamous metaplasia[7].

Although inhaled corticosteroids are the most useful anti-inflammatory drugs for the therapeutic management of chronic obstructive bronchitis, there is a large body of evidence that theophylline may be valuable at reducing inflammation of the airways at concentrations which are therapeutically relevant[8], which is not the case with beta-2-stimulant agents[9]. The effectiveness of theophylline in attenuating antigen-mediated early

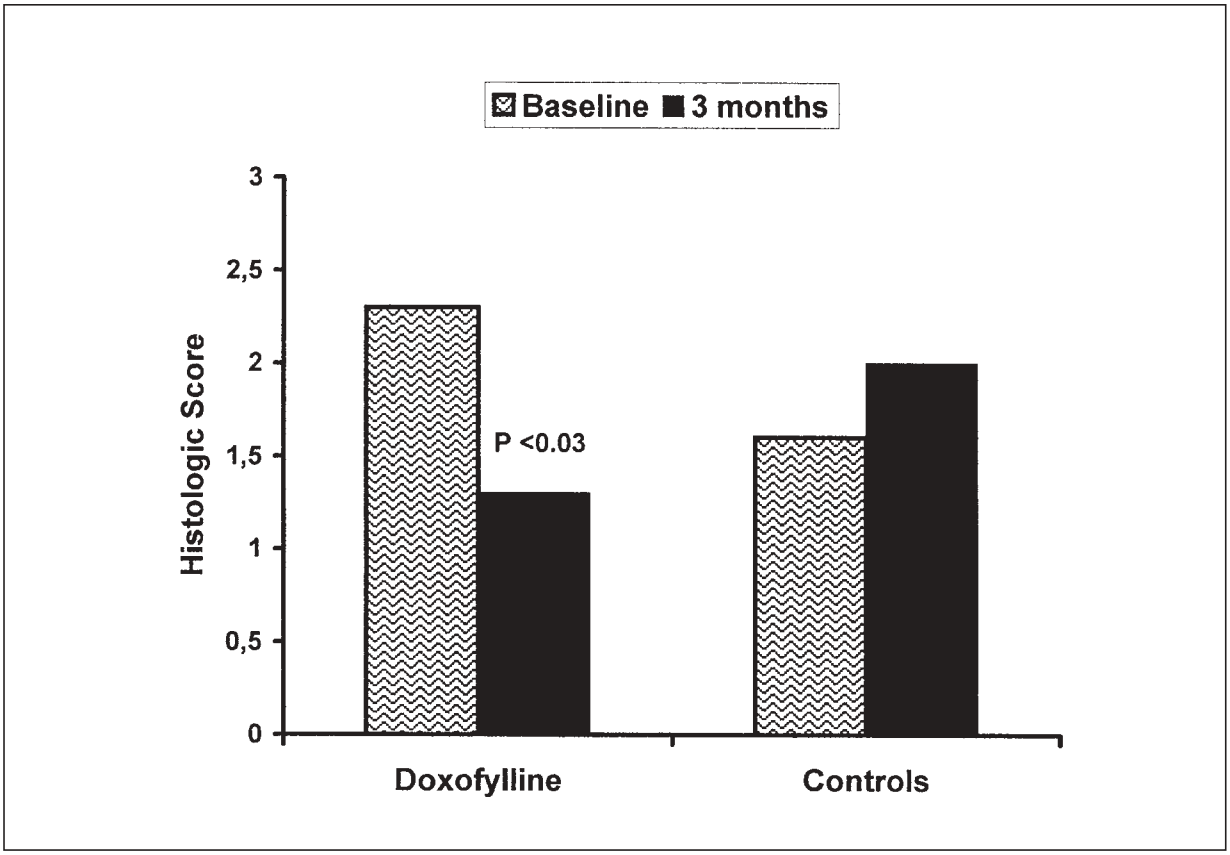

Figure 2. Histograms displaying histologic scores from bronchial wall specimens in the group of patients treated with doxofylline and in the control group at baseline and after three months.

and late hyperresponsiveness of the airways has been recently demonstrated in comparison with steroids and leukotriene-antagonists[10]. Moreover, it has been recognized that theophylline may improve mucociliary clearance, which is known to be depressed in patients with chronic obstructive bronchitis[11,12].

With the introduction of doxofylline – a methylxanthine of the last generation – studies have been performed not only to prove its bronchodilator activity but also to test the anti-inflammatory effects of the drug[2]. In clinical trials carried out in patients with asthma or chronic obstructive bronchitis, treatments with oral doxofylline – from 400 mg BID to 400 mg TID – were associated with 13 to 33% improvements in FEV_1[13-15]. The results of the studies performed in asthmatic patients showed that doxofylline was as effective as theophylline while it was superior than placebo in relieving symptoms associated with airway obstruction[11,15,16]. In these studies, the number of side effects with doxofylline was little more than those of placebo, but significantly less compared to theophylline[13,15].

In the present study, patients that improved clinically had a regression of structural and inflammatory wall changes despite the lack of significant improvements in lung function. Specimens from most of the patients with chronic bronchitis treated with doxofylline (57%) showed a remarkable reduction in bronchial airway inflammation and neutrophilic infiltration. Moreover, none of the patients receiving doxofylline exhibited replacement of ciliated columnar cells by squamous and goblet metaplasia. Marked improvements in histological lesions were reported in only 14% of the samples of patients of the control group.

Possible direct anti-inflammatory actions of doxofylline are supported by a number of experimental and clinical observations. In vitro studies showed a significant protective effect exerted by the drug against platelet activating factor (PAF)-induced bronchial airway inflammation[17]. In asthmatic patients, the protective action of doxofylline against airway inflammation was demonstrated by the inhibitory effects against the allergenic challenge induced by dermatophagoides[18]. Finally, possible direct effects of the drug on the release of histamine and leukotrienes have been hypothesized based on the efficacy of intravenous doxofylline in reducing airways resistances in patients with adult respiratory distress syndrome (ARDS)[19].

Inasmuch airway inflammation may be the major cause of symptoms in chronic bronchitis, the relationship between clinical efficacy and the reduction of structural changes and altered cell proliferation in the doxofylline group may reflect anti-inflammatory and mucus-modulating properties of the drug[20]. In addition, the decrease in plasma exudation and mucus secretion following the administration of doxofylline may contribute to the improvement in mucociliary clearance of the respiratory tract.

There are a number of limitations in the study that merit to be considered before drawing the final remarks. First, a definite demonstration of the effect of the drug on inflammatory markers is still lacking. Second, because of the small number of patients involved in the study, there is a possibility of an elusive association between doxofylline and the regression of inflammatory changes during the treatment. Even though the latter represents the major limitation of the study, the trend toward a worsen histology in the control group may reflect a higher degree of inflammation of the small airways, that may be explained because of the absence of any anti-inflammatory treatment.

In conclusion, the observations of the present study suggest that the potential usefulness of doxofylline in chronic obstructive bronchitis may descend not only from its bronchodilator activity but also from its ability to reduce inflammatory changes and altered cell proliferation of the bronchial wall. However, further study is required to better examined the long-term effect of doxofylline on airway inflammation, mucus secretion and mucocilary clearance.

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