Original Research Article

A prospective randomized controlled study: Theophylline on oxidative stress and steroid sensitivity in chronic obstructive pulmonary disease patients

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Abstract

Objective: Oxidative stress is involved in the pathogenesis of chronic obstructive pulmonary disease (COPD). Corticosteroid fails to suppress inflammation and oxidative stress due to steroid resistance. Theophylline has an effect on histone deacetylase (HDAC) activity and improves steroid sensitivity in COPD. Given changes in oxidative stress associated with diminished corticosteroid effects, a clinical study in which antioxidants and free radicals are estimated can suggest a correlation between antioxidants, theophylline, and corticosteroid sensitivity.

Materials and Methods: A randomized controlled study was conducted in 60 participants divided into 4 groups: Group I (controls) - 15 normal healthy volunteers, Group II - COPD patients who received theophylline 300 mg + salbutamol 8 mg, Group III - patients who inhaled budesonide 400 μg + salbutamol 8 mg, and Group IV - theophylline 300 mg + inhaled budesonide 400 μg + salbutamol 8 mg 12 weeks. Blood samples were collected at the time of diagnosis and at 4-week interval for 3 months from all the groups and antioxidant parameters, spirometric % forced expiratory volume in 1 s (FEV_1) were measured.

Results: The mean difference between groups was analyzed using one-way ANOVA. There was a significant increase in antioxidant enzymes such as catalase, glutathione (GSH) serum transferase, ($P < 0.05$), reduced GSH, and superoxide dismutase ($P < 0.01$) and a significant decrease in lipid peroxidation ($P < 0.01$) at 12 weeks of the study period. Postbronchodilator FEV_1 values have also shown a significant increase at 12 weeks ($P < 0.01$).

Conclusion: Theophylline increases the expression and activity of HDAC and improves steroid sensitivity thereby decreases oxidative stress. Hence, novel therapeutic strategy is therefore the reversal of this corticosteroid resistance by increasing the expression and activity of HDAC achieved using corticosteroids along with theophylline.

Keywords: Antioxidant, forced expiratory volume, histone deacetylase, lipid peroxidation protease-antiprotease

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INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a major health problem worldwide that has an increasing prevalence and mortality. It is a preventable and treatable disease with some significant extra-pulmonary effects that may contribute to the severity in individual patients.

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Its pulmonary component is characterized by airflow limitation that is not fully reversible. The airflow limitation is usually progressive and associated with an abnormal inflammatory response of the lung to noxious particles or gases. Hence, COPD is characterized by inflammation and oxidative stress.[1] The lungs are continuously exposed to oxidants generated exogenously from air pollutants or cigarette smoke and endogenously from phagocytes and mitochondrial electron transport chain that are involved in many cellular signaling pathways. Protection against this oxidative challenge is mediated by well-developed enzymatic systems such as antioxidants and nonenzymatic systems that include first-line defense mechanisms through cellular processes such as phagocytosis mediated by neutrophils, macrophages, and fluid lining respiratory tract. Oxidative stress is a predominant etiopathological basis of COPD.[2]

Oxidative stress results from an imbalance between the continual endogenous production of reactive oxygen species (ROS) and naturally occurring antioxidants. Oxidative stress is an etiological factor in many diseases including neurodegenerative diseases, adult respiratory distress syndrome (ARDS), atherosclerosis, heart failure, diabetes mellitus, and cancer an arthritis. The ROS also known as free radicals include superoxide anion (O^{2-}), hydrogen peroxide, peroxynitrite ($ONOO^{-}$), and others. Naturally occurring antioxidants include catalase, superoxide dismutase (SOD), glutathione (GSH), GSH-s-transferase (GST), and Vitamins A, C, and E.[3]

Prevalence rates vary from country to country, largely on the basis of exposure to risk factors, average age of the population, and increases with age. The World Health Organization has estimated the global prevalence of COPD to be about 0.8% of the world population, with somewhat higher rates in men (0.9%) than in women (0.7%). Mortality statistics for COPD show an increasing trend with increase in age and also smoking increases mortality in COPD patients.[4] Many risk factors contribute to the development of COPD, these factors include environmental issues (humidity, dust, hot weather, very cold weather, etc.,) genetic susceptibility, age >40 years, gender, nutrition, socioeconomic status, oxidative stress, occupational hazards, cigarette smoking, fuel fumes, and many others.[5]

Alveolar macrophages present in lungs produce histone deacetylase (HDAC) which suppresses the production of cytokines that mediate inflammation. HDAC2 is a Class I HDAC family member that plays a critical role in suppressing inflammatory gene expression in the airways, lung parenchyma, and alveolar macrophages in patients with COPD. This HDAC activity is reduced in patients with COPD. Besides inflammation, two other processes are involved in the pathogenesis of COPD - an imbalance between proteases and antiproteases and an imbalance between oxidants and antioxidants (oxidative stress) in the lungs**.** Increased activity or production of proteases and inactivation or reduced production of antiproteases results in imbalance.[6,7] The main proteases involved are those produced by neutrophils (including the serine proteases such as elastase, cathepsin G, and protease-3) and macrophages (cysteine proteases and cathepsin E, A, L, and S), and various matrix metalloproteases.[8,9]

Conventionally, patients with COPD have been treated with theophylline and salbutamol which are bronchodilators and corticosteroids, an anti-inflammatory drug and the clinical improvement is assessed using spirometric forced expiratory volume in 1 s (FEV_1) parameter. High-dose corticosteroids administered at the time of exacerbations reported to reduce the exacerbations. Corticosteroids reduces the severity of acute respiratory distress syndrome (ARDS) and improves lung function.[10,11] The anti-inflammatory effects of corticosteroids are increased along with steroid sensitivity when given with theophylline, probably due to change in the acetylated status of histone in alveolar macrophages as suggested by *in vitro* studies. This potentiation of the anti-inflammatory effect due to corticosteroids could be due to increased steroid sensitivity that can be attributed to theophylline. It has been known that theophylline at its therapeutic concentrations activates HDAC resulting in increased steroid sensitivity.[12,13]

Thus, COPD is associated with inflammation and increased oxidative stress. The decrease in the symptoms of COPD when treated with corticosteroids and theophylline following enhancement of anti-inflammatory effect suggests a role for corticosteroids in this outcome and probably the resulting decrease in oxidative stress. The present study was done to investigate the role of theophylline on steroid sensitivity and oxidative stress in COPD patients.

MATERIALS AND METHODS

The study was conducted after having obtained Ethical clearance from Human Ethical Committee at Rajiv Gandhi Government General Hospital, Madras Medical College, Chennai. Informed consent in the prescribed form was obtained from all patients included in the study after explanation of the probable cons and pros in local language.

Study design and participants

A randomized controlled study was conducted in sixty participants in which 15 healthy volunteers (Group I - controls) and 45 patients of COPD diagnosed according to global initiative for chronic obstructive lung disease (GOLD) guidelines are identified and divided into three groups of 15 patients in each group and compared with control group. In Group II, COPD patients received theophylline 300 mg + salbutamol 8 mg, Group III patients inhaled budesonide 400 μg + salbutamol 8 mg, and Group IV patients received theophylline 300 mg + inhaled budesonide 400 μg + salbutamol 8 mg for 12 weeks. Blood samples were collected at the time of diagnosis and up to 12 weeks at the interval of 4 weeks for all the groups except for the control group and spirometric % FEV_1 parameter was measured. The study design is shown in [Figure 1].

Inclusion criteria

All new patients referred to the thoracic medicine outpatient clinic with stable COPD were recruited for the study according to the GOLD guidelines for COPD. %FEV_1 (FEV_1/[forced vital capacity (FVC)]) is the gold standard for confirming the diagnosis of COPD.[14] The patients selected had FEV_1/FVC of 70% or less and also they were categorized under moderately ill patients as indicated by FEV_1 of 50%–80% predicted. All the patients were subjected to inhalation of salbutamol 2.5 mg by nebulizer and postbronchodilator FEV_1 was measured. It is important to ensure that patients have not taken any bronchodilators before and to differentiate COPD from asthma.

Exclusion criteria

Patients those who are not willing to give consent, patients who also ill for a complete follow-up, patients with other concurrent major respiratory diseases other than COPD, patients with a history of diabetes mellitus, patients with a history of cardiac failure, and patients with a history of hepatic disease and renal disease were excluded from the study.

Blood collection and laboratory methods

Blood sample was collected under aseptic precautions at the time of diagnosis and at 4-week interval for 12 weeks. The biochemical profile was evaluated using standard laboratory methods. Samples were kept cool until the blood collection and then centrifuged at 4000 rpm, at 4°C for 5 min. The serum was separated and analyzed for catalase (method of Sinha., 1972), reduced GSH (method of Ellman., 1959), GST (method of Habig *et al.*, 1974), SOD (method of Marklund and Marklund., 1974), and free-radical production were measured by lipid peroxidation (LPO) (method of Ohkawa *et al.*, 1979). Protein content was measured by the method of Lowry *et al.* (1951). All these parameters were determined using ultraviolet spectrophotometer.

Statistical analysis

The data were presented as mean ± standard deviation. Comparison of data between groups was performed using one-way ANOVA. Data analysis was performed with statistical package for social sciences version 20.0 (Armonk NY : IBM Corp) for Windows. $P < 0.05$ was considered statistically significant.

RESULTS

Antioxidants

When compared from 0 to 12 weeks, there is a significant increase in all antioxidants catalase, GST ($P < 0.05$), GSH, SOD ($P < 0.01$), and LPO showed a significant decrease from 0 to 12 weeks ($P < 0.01$). When compared to Group III, Group IV has shown a significant increase in GSH level ($P < 0.05$) at 4th, 8th week, and more significant increase ($P < 0.01$) at 12th week of the study period (10.88 ± 0.87). When compared to Group III, Group IV has shown a significant increase in GST level ($P < 0.01$) at 8th week (13.77 ± 0.64) and 12th week (13.81 ± 0.61) of the study period [Table 1]. When compared to Group III, Group IV has shown a significant increase in SOD level ($P < 0.01$) at 12th week (2.21 ± 0.26) of the study period [Table 1]. When compared with control group, all groups have shown a significant increase in LPO level ($P < 0.05$) at 4th, 8th, and 12th week of the study period. When compared to Group III, Group IV has shown a significant decrease in LPO level ($P < 0.01$) at 4th week, 8th week, and 12th week of the study period. When compared to Group III, Group IV has shown a significant decrease in LPO level ($P < 0.01$) at 12th week (3.33 ± 0.07) of the study period.[Table 1].

Forced expiratory volume

Postbronchodilator FEV_1 when compared with baseline values has shown a significant increase ($P < 0.01$) in FEV_1 values at 4th week, 8th week, and 12th week of the study period. However, there was no significant difference between the patient groups at any period of the study [Table 1].

DISCUSSION

COPD is the fourth of global burden disease worldwide. The main pathology is due to inflammatory and cellular responses due to cigarette smoke exposure. COPD leads to two major clinical conditions: (a) bronchitis associated

Table 1: Antioxidant enzymes and post bronchodilator forced expiratory volume in 1 s parameters among groups

Enzymes	Duration	Group I	Group II	Group III	Group IV
Catalase	0th week	84.03±9.79	58.75±5.36	59.85±5.37	59.86±4.18
	4th week	84.12±9.79	60.65±3.94[a,**]	60.20±5.01[a,**]	61.23±3.90[a,**]
	8th week	83.99±9.79	61.01±3.73[a,**]	60.54±4.61[a,**]	61.99±3.59[a,**]
	12thweek	84.13±9.79	61.85±2.99[a,**]	60.66±4.37[a,**]	62.15±2.99[a,**]
Reduced glutathione	0th week	12.64±0.94	9.95±1.44	9.79±0.92	10.55±1.19
	4th week	12.43±0.94	10.34±1.12	9.93±0.79	10.74±1.03[b,*]
	8th week	11.94±0.94	10.47±1.03	10.05±0.77	10.82±0.92[b,*]
	12thweek	12.68±0.94	10.58±0.93	10.07±0.72	10.88±0.87[b,**]
Glutathione S transferase	0th week	19.50±2.45	13.08±0.99	12.87±1.11	13.27±1.23
	4th week	19.61±2.45	13.30±0.90[a,**]	13.02±0.94[a,**]	13.50±1.13[a,**]
	8th week	19.43±2.45	13.33±0.79[a,**]	13.11±0.91[a,**]	13.77±0.64[a,b,**]
	12thweek	19.70±2.45	13.46±0.69[a,**]	13.22±0.73[a,**]	13.81±0.61[a,b,**]
Superoxide dismutase	0th week	2.70±0.18	1.86±0.30	1.82±0.42	1.72±0.43
	4th week	2.65±0.18	2.04±0.28	1.94±0.32	2.08±0.36
	8th week	2.78±0.18	2.07±0.25	2.03±0.32	2.12±0.40
	12th week	2.50±0.18	2.14±0.30	2.03±0.29	2.21±0.26[b,**]
Lipid peroxidation	0th week	2.72±0.23	3.81±0.56	4.20±0.60	3.88±0.62
	4th week	2.69±0.23	3.75±0.53[a,*]	4.06±0.50[a,*]	3.62±0.56[a,b,*]
	8th week	2.78±0.23	3.65±0.46[a,*]	3.97±0.48[a,*]	3.48±0.46[a,b,*]
	12th week	2.67±0.23	3.52±0.38[a,*]	3.89±0.45[a,*]	3.33±0.27[a,b,*]
Postbronchodilator FEV_1	0th week		66.47±7.80	69.47±6.27	69.87±6.52
	4th week		67.60±7.78[a,*]	69.67±6.08[a,*]	70.13±6.11[a,*]
	8th week		69.73±7.11[a,*]	70.33±5.83[a,*]	71.20±5.89[a,*]
	12th week		70.87±7.21[a,*]	70.47±5.55[a,*]	72.53±5.51[a,*]

[a]Compared with controls, [b]Group III compared with Group IV, $**P<0.01$, $*P<0.05$. FEV_1: Forced expiratory volume in 1 s

with airway inflammation and mucus obstruction and (b) emphysema characterized by loss of alveolar surface area for gas exchange.[15,16] Oxidative stress is also one of the most important mechanisms in amplifying inflammation in COPD. Oxidative stress parameters are increased in sputum, systemic circulation, and exhaled breath of COPD patients.[17] During exacerbations, oxidative stress is further increased. The main sources of oxidants are cigarette smoke and other inhaled particles.[18] Endogenous antioxidants may also be reduced in COPD patients.[19,20] Oxidative stress in lungs leads to activation of inflammatory genes, mucus secretion, and inactivation of antiproteases. Oxidative stress also alters histone acetylation and deacetylation which leads to increased proinflammatory mediators in lung.[21,22] Since oxidative stress is involved in pathogenesis, there are controversies regarding treatment with antioxidants in COPD. One previous study stated that ascorbic acid has no established bronchodilatory or anti-inflammatory effect.[23]

HDAC is a key molecule in the repression of production of proinflammatory cytokines in alveolar macrophages, and it has been recently discovered that HDAC activity is reduced in lungs of COPD patients which accounts for amplified inflammation and steroid resistance. Inflammation in COPD is mainly mediated by an increased expression of various inflammatory genes. The increased expression of these genes is regulated by acetylation of core histones, whereas HDAC2 suppresses inflammatory gene expression. In COPD, there is an increase in acetylation of histones associated with the promoter region of inflammatory genes such as interleukin - 8 (IL-8) and tumor necrosis factor-α (TNFα) that are regulated by nuclear factor kappa B (NF-kB) (transcription gene). The degree of acetylation increases with disease severity. This increased acetylation is due to decrease in HDAC.[24]

There are 11 isoforms of HDAC which reverses histone acetylation by suppression of inflammatory gene expression. HDAC activity is lost in peripheral lung, alveolar macrophages of COPD patients due to increased oxidative stress.[25] Nitric oxide (NO) and O^{2-} generated by cigarette smoke and inflammatory cells combine to form $ONOO^-$. These $ONOO^-$ nitrates HDAC mainly at the tyrosine residue within the catalytic site results in reduced expression of HDAC. Hence, there is relative increase in histone acetyltransferase which increases histone acetylation and resulting in the blockage of effects of glucocorticosteroids. HDAC function is thus restored by theophylline, antioxidants, and inducible NO synthase inhibitors (generates NO due to smoke).[26,27]

Normal lung when stimulated by irritants activates NF-kB to switch on histone acetyltransferase which leads to histone acetylation and also increases in inflammatory mediators such as IL-8 and TNF-α. Glucocorticosteroids reverse this by binding to its receptor and recruiting HDAC. In COPD patients because of increased oxidative stress, there is impairment of HDAC. This amplifies the inflammatory process and reduces the effects of glucocorticosteroids.

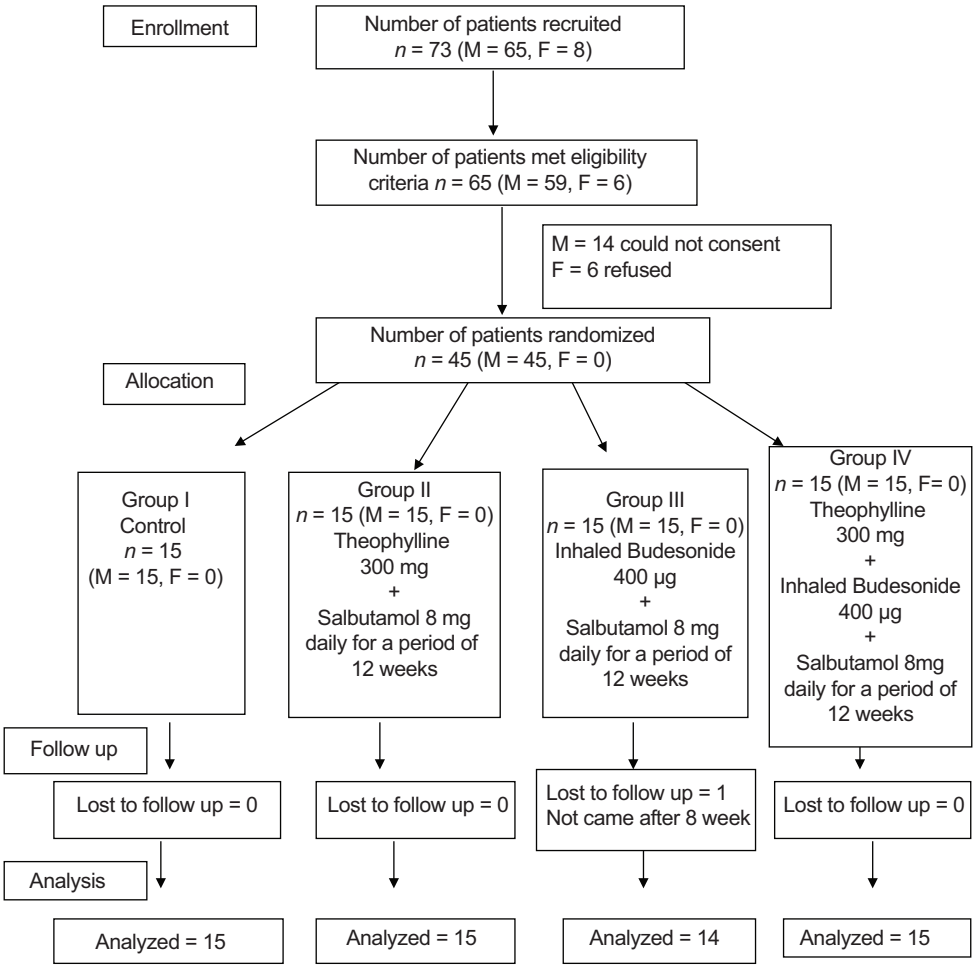

Figure 1: Requirement, allocation, and follow-up of participants

Treatment with drugs has resulted in an improvement in the antioxidant levels with declining free-radical production. A significant decrease in free-radical production in Group IV, compared with Group II, indicates that addition of steroid results in better performance (i.e., reduction in free-radical production). Corticosteroid resistance in COPD occurs because corticosteroids use HDAC2 to switch off activated inflammatory genes. The reduction in HDAC2 appears to be secondary to the increased oxidative and nitrative stress in COPD lungs. Antioxidants and inhibitors of NO synthesis may therefore restore corticosteroid sensitivity in COPD, but this can also be achieved by low concentrations of theophylline and curcumin, which act as HDAC activators. Two small randomized controlled trials stated that theophylline at "low dose" (plasma concentration of 1–5 mg/l) increases the sensitivity of COPD airway inflammation to the anti-inflammatory effects of inhaled corticosteroids. Previous studies have investigated the potential anti-inflammatory effects of "low dose" theophylline in COPD and asthma (not in conjunction with inhaled corticosteroids). The demonstration that low-dose theophylline increases the efficacy of inhaled corticosteroids in COPD by reducing the exacerbations is relevant not only to patients and clinicians but also to health-care providers globally.[28,29]

The report that theophylline improves steroid sensitivity is manifested in this study since Group IV has significant lesser serum LPO compared to Group III. Corroborating with this, an improvement was also seen in antioxidants as evidenced by GSH (after all duration of treatment), GST at 8 weeks, and SOD at the end of 12 weeks. Improvement in antioxidant levels and reduction in free-radical production could be regarded as an increase in corticosteroid sensitivity due to treatment with theophylline. This could be due to the effect on HDAC as cited in the literature reviews. This is in agreement with the *in vitro* reports that theophylline improves steroid sensitivity. Recruitment of HDAC by glucocorticoid receptors switches off inflammatory genes. Therapeutic concentrations of theophylline activate HDAC, thereby overcome the insensitivity to steroids. This mechanism is independent of phosphodiesterase inhibition or adenosine receptor antagonism and appears to be mediated by the inhibition of PI3-kinase-δ, which is

activated by oxidative stress. Hence, low-dose theophylline may "unlock" the glucocorticoid resistance of COPD and potentiate its suppressive effects.

CONCLUSION

The observation made in COPD patients that theophylline improved steroid sensitivity can have therapeutic implications. Inhaled corticosteroids even in high doses fail to suppress inflammation and also reactive oxygen intermediates in COPD, this is because of active resistance mechanism linked to a reduction in HDAC. Hence, novel therapeutic strategy is therefore the reversal of this corticosteroid resistance by increasing the expression and activity of HDAC. This is achieved using corticosteroids along with theophylline, antioxidants, and antibiotics such as macrolides.

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Conflicts of interest
There are no conflicts of interest.

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