Chronic obstructive pulmonary disease (COPD) is one of the most frequent diseases worldwide. Cigarette smoke is considered the main pathological cause of the disorder, although evidence is growing concerning other etiological factors, such as environmental pollution, biomass combustion, infections, genetic predisposition, which may explain why some individuals develop COPD with no history of smoking. Chronic inflammation and remodeling of the small airways characterize the disease at the cellular level, and oxidative stress is considered the main driving force that stands behind COPD inflammation. Recently, chromatin remodeling and epigenetic changes have been found to underlie disease pathology and progression. In this review, the authors gave a short update on the recent hypothesis and findings that may imply novel approach to pharmacotherapy of the disease, focusing on the role of glucocorticosteroids, theophylline, and antioxidants.

**Key words:** COPD, glucocorticosteroids, HDAC, transcription factors

**INTRODUCTION**

COPD is defined as a preventable and treatable disease with some significant extrapulmonary effects that may contribute to severity in individual patients (1). The global prevalence in adults aged over 40 is estimated to be 9–10%. There is a prediction that COPD will become the fifth most frequent burden of disease worldwide (2). The pulmonary component of COPD is characterized by airflow limitation that is not fully reversible, but is usually progressive and associated with an abnormal inflammatory response of the lung to noxious particles or
gases. Pathologically, easily visible disease processes are chronic bronchitis, emphysema, and small airways disease. Cigarette smoke is considered the main pathological cause of the disorder, though evidence is growing on other factors, such as environmental pollution, biomass combustion, infections, and genetic predisposition, which may explain why some individuals develop COPD with no history of smoking.

Emphysema is an anatomopathological diagnosis, which is defined by a permanent destructive enlargement of airspaces distal to the terminal bronchioles, resulting from loss of lung elastic recoil and having impact on airflow limitation (3). Chronic inflammation and remodeling of the small airways characterize the disease at the cellular level (4). Oxidative stress is considered the main driving force that stands behind COPD inflammation (5).

EPIGENETIC CHANGES IN CHROMATIN STRUCTURE

Chromatin structure is composed of nucleosomes, which consist of approximately 146 pairs of DNA associated with an octamer of 2 molecules each of core histone proteins (H2A, H2B, H3, and H4). A characteristic feature of histones is the large number and type of modified residues they possess. In the resting cell, DNA is tightly compacted around the basic core histones, preventing the binding of the enzyme RNA polymerase II, which activates the formation of messenger RNA. Expression and repression of genes is associated with alterations in chromatin structure by postranslational modification of core histones (6, 7). The surface of nucleosomes is studded with a multiplicity of modifications, representing an additional level of control in numerous nuclear processes, such as transcriptional regulation, replication, recombination, and DNA damage repair. Modulations of chromatin structure that accompany transcriptional regulation often require multiprotein complexes that can manipulate the nucleosomal architecture. Acetylation and phosphorylation, are two highly conserved chromosome-modifying enzymatic activities best so far studied, and the number of types and sites of these epigenetic changes is still growing in the literature, including methylation, ubiquitylation, sumoylation, deimination, proline isomerisation, and/or ADP ribosylation (8, 9). The number of histone residues which can be modified is accounted to be over 60 and this number represents a huge underestimate due to extra complexity which comes partly from the fact that methylation at lysines or arginines may be one of three different forms: mono-, di-, or trimethyl for lysines and mono- or di- (asymmetric or symmetric) for arginines, leading to an enormous potential for functional responses, which can be mediated through postranslation histone modification (8). These modifications function either by disrupting chromatin contacts or by affecting the recruitment of non-histone proteins to chromatin. Their presence on histones can dictate the higher-order chromatin structure in which DNA is
packaged and can orchestrate the ordered recruitment of enzyme complexes to manipulate DNA. In this way, histone modifications have the potential to influence many fundamental biological processes, some of which may be epigenetically inherited (8). Kouzarides (8) divides the function of histone modifications into two categories: the establishment of global chromatin environments and the orchestration of DNA-based biological tasks. A global chromatin environment is established by modifications leading to active euchromatin, where DNA is kept ‘accessible’ for transcription, and silent heterochromatin, where chromatin is ‘inaccessible’ for transcription (8, 10). DNA-based functions are facilitated by modifications which unravel chromatin to allow a specific function, from a very local one, such as transcription of a gene, cell proliferation or the repair of DNA or it may be a more genome wide function, such as DNA replication or chromosome condensation (8).

CHROMATIN REMODELING IN COPD

Epigenetic changes have not been well studied in human airways disease. Data focussed on the remodeling of chromatin and transcription factors activation during the course of asthma and COPD come mainly from the group of Ito et al (11), who for the first time reported decreased HDAC2 expression and activity in lung macrophages, biopsies, and blood cells from patients with COPD, severe asthma, and smokers with asthma. Histone acetylation is reversed by histone deacetylases (HDACs). Recent studies suggest that HDACs, interacting with corepressor molecules, such as nuclear receptor corepressor, ligand-dependent corepressor, NuRD, and mSin3 act to repress the expression of inflammatory genes (reviewed in 12). HDACs play also a role in regulation of transcription activity of other factors like GATA3 and the p-65 component of NF-κB, without altering DNA binding process. CBP acetylates specific lysine residues on p65, increasing its binding to DNA and causing transcriptional activation. HDACs reverse this process by removing acetyl groups from hyperacetylated NF-κB and promote its association with the inhibitor IκF-0αB within the nucleus and terminate the activity of NF-κB (13). Moreover, inhibition of HDAC1 and HDAC2 by trichostatin increases activation of NF-κB; hence increases the expression of inflammatory genes such as IL-8 (14). The expression of inflammatory genes is mainly determined by a balance between histone acetylation and deacetylation (12).

TRANSCRIPTION FACTORS IN COPD

The data on the role of transcription factors in inflammation is substantially growing. Transcription factors play a key role in the regulation of cell function, growth, and differentiation. Moreover, they may also play a pivotal role in
chronic inflammatory diseases (15). According to Barnes (16), one of the most important concepts that have recently emerged is that transcription factors may interact with other transcription factors, which then allows a cross-talk between different signal transduction pathways at the level of gene expression. This interaction is particularly relevant to the action of drugs, such as glucocorticoids and cyclosporin A that activate transcription factors that subsequently modulate other transcription factors (16). Each individual modification on histones leads to a biological consequence. However, a proof of a consequence is not always easy to provide and is often based on a correlation: a modification appears on a gene under certain conditions (e.g., when it is transcribed) and disappears when that state is reversed (e.g., when the gene is silent) (8). The key transcription factors involved in airway diseases are the nuclear factor-kappa B (NF-κB) and the activator protein-1 (AP-1) (17, 18). NF-κB is the best studied transcriptional regulator of several inducible genes, such as cyclooxygenase (COX-2), nitric oxide synthase (iNOS), interleukin (IL-8), eotaxin, and adhesion molecules, such as ICAM-1 and VCAM-1, all playing a key role in inflammatory cell recruitment (16). NF-κB, present in the cytoplasm in an inactive form may be activated by oxidants, such as hydrogen peroxide (H₂O₂) and may thus function as an oxidative-stress responsive transcription factor. This may be relevant in chronic inflammation when oxidants, such as superoxide anions are generated by inflammatory cells and in asthma, where inhalation of environmental oxidants, such as ozone, may amplify inflammation (16). Activator protein-1 (AP-1), a collection of related transcription factors belonging to the Fos (c-Fos, FosB, Fra1, Fra2) and Jun (c-Jun, JunB, JunD) families, may be activated via PKC and by various cytokines, including TNF-α and interleukin (IL-1β), via several types of protein tyrosine kinase (PTK) and mitogen activated protein (MAP) kinase, which themselves activate a cascade of intracellular kinases (16, 18).

GLUCOCORTICOSTEROID RECEPTOR MODE OF ACTION AND RECENT HYPOTHESIS ON GLUCOCORTICOSTEROID RESISTANCE IN COPD

Glucocorticosteroids (GCS) bind to specific cytosolic glucocorticosteroid receptors (GR), which are held in a resting state by a number of chaperone proteins. After translocation to the nucleus, the activated GR can induce the expression of a number of key anti-inflammatory genes following a direct association with DNA at GCS response elements (GREs) in the promoter regions of these genes, or the activated GR can selectively repress the transcription of specific inflammatory genes without binding to DNA itself, but by a number of pleiotropic actions at the promoters of inflammatory genes (19). Following its activation, GR binds to transcription factors, such as NF-κB or AP-1, either directly or indirectly, and recruits corepressor proteins that blunt the ability of these transcription factors to switch on inflammatory genes (20). GCS resistance
which may occur in the course of asthma and COPD has been variably ascribed to reduced GR expression, altered affinity of the ligand for GR, reduced ability of the GR to bind to DNA, reduced expression and/or activity of corepressor proteins, or increased expression of inflammatory transcription factors, such as NF-κB and AP-1 (19). Ito et al (11) reported that specimens of lung tissue obtained from patients with increasing clinical stages of COPD have graded reductions in HDAC activity and increases in IL-8 messenger RNA (mRNA) and histone-4 acetylation at the IL-8 promoter. The mRNA expression of HDAC2, HDAC5, and HDAC8 and expression of the HDAC2 protein are also lower in patients with increasing severity of the disease. HDAC activity was decreased in patients with COPD, as compared with normal subjects, in both macrophages and biopsy specimens, with no changes in HAT activity, whereas HAT activity was increased in biopsy specimens obtained from patients with asthma (11). According to Barnes (16) and Barnes et al (21), decreased HDAC activity may be due to inactivation of the enzyme of oxidative and nitrative stress. Furthermore, authors hypothesize that oxidative and nitrative stress lead to the formation of peroxynitrite, which nitrates tyrosine residues on certain proteins. A high level of oxidative/nitrative stress in the COPD lungs may result in increased tyrosine nitration and impaired HDAC2 function and a reduction in its expression, which leads to increased expression of inflammatory genes and impaired responses to glucocorticoids (21). Cigarette smoke also reduces HDAC2 activity and this may explain why asthmatic patients who smoke have a markedly reduced response to glucocorticoids (21).

In our previous paper, we have described increased expression and activation of nuclear cyclic AMP-response element binding protein (CREB) in COPD patients treated with inhaled corticosteroids (ICS) (22). We have drawn on these mechanisms to derive our hypothesis that CREB activation can shift pro/antiinflammatory balance toward inflammation and account for a poor response of COPD patients to glucocorticoid therapy (22). Moreover, in this issue we report an increase of nuclear cAMP response element binding protein (CREB; protein and mRNA) and peroxisome proliferator-activated receptor gamma (PPARγ protein and mRNA) levels in induced sputum cells derived from formoterol/GCS-treated COPD patients. CREB-binding protein (CBP; protein and mRNA) levels were significantly lower in formoterol/ICS-treated COPD patients (23). We did not detect altered 8-isoprostane levels in COPD patients during formoterol or formoterol/ICS therapy, but a reduction of oxidative stress as a result of addition of theophylline to formoterol /ICS therapy has been reported by others (24). To further characterize alterations in nuclear signaling in COPD patients subjected to glucocorticoid therapy, we examined transcriptional co-integrator CBP, which binds CREB and mediate anchoring of proinflammatory NF-κB and AP-1 molecules (15). The main signaling pathway of glucocorticoids is related to GR activation and transcriptional repression, thus interactions between activated GR and inflammatory signaling molecules like
NF-κB, CREB, and AP-1 are very important. Our data indicate that in formoterol/ICS treated patients, GR protein expression and GR mRNA levels are not significantly different from the corresponding levels in formoterol-treated patients, while the nuclear CREB and its mRNA are elevated. However, CBP mRNA and protein are significantly lower in formoterol/ICS-treated patients compared with formoterol-treated patients and this may result in decreased CREB-mediated signaling (23).

Up-to-date, the well known molecular mechanisms of GCS resistance found in subpopulation of asthma and COPD patients are: reduced GR expression, altered affinity of the ligand for GR, reduced ability of the GR to bind to DNA, reduced expression and/or activity of corepressor proteins, or increased expression of inflammatory transcription factors, such as NF-κB and AP-1 (19-21), some of which may influence the treatment outcome in COPD patients. Oxidative stress generated by reactive oxygen species contained in cigarette smoke leads to altered GR function, including nuclear translocation (25). Therefore, COPD patients and smokers with asthma may benefit from the treatment with antioxidants (25). Impaired nuclear translocation of GR may further be enhanced with LABA treatment (19). High levels of nitric oxide (NO) from cigarette smoke may lead to altered ligand binding through GR nitrosylation at an hsp90 interaction site (19). Rahman and Adcock (25) suggested that GCS insensitivity caused by conversion of NO to peroxynitrate may be effectively treated with NO synthase NOS-2 inhibitors. Normally, GR recruits corepressor proteins, such as histone deacetylase (HDAC) 2, to actively transcribing gene complexes within nucleus, which leads to the suppression of proinflammatory genes (19), reviewed in (20). As reviewed by Adcock and Barnes (20), reduced HDAC2 activity in BAL fluid macrophages from smokers inversely correlates with GC sensitivity. HDAC2 expression and activity are further reduced in BAL fluid macrophages, bronchial biopsy specimens, and peripheral lung tissue from patients with COPD and in the peripheral blood cells of asthmatic patients who smoke compared with non-smokers (reviewed in 20). Rahman and Adcock (25) suggested that in COPD patients the suppression of HDAC2 activity may be due to tyrosine nitration, again implicating a potential therapeutic role for antioxidants (23). Cosio et al (24) suggested that impaired HDAC2 activity characterizing macrophages derived from COPD patients may be effectively restored by add-on therapy with GCS/theophylline in patients with severe asthma and COPD and that this mechanism of an enhancement HDAC2 activity is independent of theophylline’s bronchodilator actions or inhibitory effects on phosphodiesterase-4 activity (24). Therefore, the add-on therapy using GCS and theophylline is strongly suggested (20). As we stated before in our previous paper, we report that the treatment with formoterol/GCS increased the expression of CREB and CREB-P in both cytosolic and nuclear fractions obtained from induced sputum of COPD patients. These changes are not affected by theophylline. Assuming that, we suggest that these findings may indicate that poor response to
ICS therapy may be related to an increase in the CREB-associated signaling (22). Our recent findings on formoterol/GCS treatment which increased nuclear cAMP response element binding protein (CREB; protein and mRNA) and peroxisome proliferator-activated receptor gamma (PPARγ protein and mRNA) in cells from induced sputum of COPD patients further supports these findings. Moreover, as stated before CREB-binding protein (CBP; protein and mRNA) levels were significantly lower in formoterol/ICS treated patients (23). Adcock and Barnes (20) suggested that the type of inflammation in GCS resistant patients with COPD and asthma may be distinct, and targeting this inflammation with selective therapeutic agents may be beneficial. Restoring GCS sensitivity rather than prevent inflammation per se is alternatively recommended. In our paper we conclude that combined formoterol/GCS therapy seems to have positive effect on basal nuclear signaling related to anti-inflammatory reactions, however it remains to be established weather similar alterations take place in lung tissue (23). Moreover, since histone modifications may be the executers of the epigenetic phenomenon rather than the carriers of the memory (8), clear mechanistic insights gained from the functional interactions between GCS, GR, corepressors and transcription factors may provide a better understanding regarding the precise role the remodeling protein plays in molecular events within the cell.

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