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DIMINISHED PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR (PPAR) REGULATION AS A POTENTIAL MECHANISM FOR THE PERSISTENT INFLAMMATION IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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WINNING ABSTRACT: Persistent inflammation is the main pathological process that underlies COPD. Understanding this inflammatory response is a key focus of COPD research with the aim of discovering new therapeutic targets. The nuclear hormone receptor, PPAR is now a recognised modulator of inflammation in various chronic inflammatory conditions, but its role in the persistent airways inflammation in COPD has not been examined. Control of the inflammatory response by PPARα has been shown by antagonising inflammatory signalling pathways, such as NF-κB and AP-1.

PPARα protein levels in lung tissue from patients with COPD were assessed by Western blot. *In vitro* assays using the human type II alveolar epithelial cell line were performed to assess the effect of PPARα agonist treatment on inflammatory cytokine generation.

An increase in PPARα protein levels was seen for healthy smokers compared with non-smokers (Ratio to β-actin loading control, non-smokers 0.61 ± 0.1, $n=10$; healthy smokers 0.97 ± 0.3, $n=11$, $p>0.05$). No increase was seen for current smoker or ex-smoker COPD patients (0.36 ± 0.08, $n=12$; 0.49 ± 0.1, $n=8$ respectively). *In vitro* experiments with a human type II alveolar epithelial cell line demonstrated a diminished inflammatory response to TNFα, as measured by the generation of the pro-inflammatory cytokine IL-8, following pre-treatment with the PPARα agonist, WY-14643 (IL-8 generation, control 823 ± 22 pg·ml^{-1}, TNFα 7491 ± 530 pg·ml^{-1} $p<0.001$, WY-14643 2559 ± 46 pg·ml^{-1} $p<0.05$, $n=3$).

We propose PPAR agonists as a potential therapy for reducing the NF-κB-regulated inflammation in COPD airways.

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MY JOB AND THE UNIT IN WHICH I WORK

As a postdoctoral fellow in the Edinburgh Lung and the Environment Group Initiative (ELEGI) Colt Laboratory (Edinburgh, UK), my research focuses on the mechanisms of lung disease caused by inhaled air pollutants. The overall aims of research carried out at ELEGI are to better understand the cellular and molecular mechanisms that lead from exposure to inflammation and lung disease, with the hope of better assessing risk or intervention in these diseases. ELEGI sits within the Centre for Inflammation Research, a large multi-disciplinary group of scientists working on a range of inflammatory diseases and mechanisms. More specifically, ELEGI research concerns the following.

1) Chronic obstructive pulmonary disease (COPD), a chronic inflammatory lung disease caused by cigarette smoking. This is a major focus of research at ELEGI, with the emphasis on the role of oxidative stress. This research comprises both clinical studies assessing biomarkers in patients with COPD to define disease phenotypes, and mechanistic research on the regulation of inflammatory genes by oxidative stress.

2) The pulmonary response to inhaled particles of all kinds. The studies aim to improve understanding of the factors that make dust and particles harmful, which will allow a more refined measurement of exposure and so improve risk management. In addition, a current area of investigation is the toxicity of nanoparticulates, in response to the rapid expansion in their use from occupational exposures to nanoparticulate-mediated drug delivery.

3) Cardiovascular disease, which is prevalent in smokers and in populations exposed to air pollution particles. Oxidative stress is hypothesised to be an aetiological factor.

MY WINNING POSTER AS PART OF MY RESEARCH

Inflammation is a prominent feature of COPD. The dominant hypothesis in the pathogenesis of COPD is that the increased oxidant burden, both directly as a result of smoking and indirectly by the release of increased amounts of reactive oxygen species from airspace leukocytes, may not be adequately counterbalanced by the lung antioxidant systems, culminating in oxidative stress. An excess of oxidants may then lead to enhanced pro-inflammatory gene expression and protein release, inactivation of antiproteases and oxidative tissue injury leading to COPD. Hence, we investigated the molecular signalling mechanisms and oxidative stress status in

lung tissue from nonsmokers and smokers with and without COPD. We hypothesised that an imbalance in histone acetylation and deacetylation accounted for the enhanced inflammatory response in ''susceptible'' smokers who develop COPD. This study showed a clear impact of cigarette smoking on chromatin remodelling (acetylation of histones 3 and 4 and histone deacetylase (HDAC)-2 levels), nuclear factor (NF)-κB-α inhibitor degradation and NF-κB translocation in lung tissue [1]. Oxidative stress, assessed by the presence of increased levels of 8-isoprostane, was increased in current smokers. Only HDAC-2 levels showed both a smoking and disease effect, which was inversely associated with disease severity.

These studies lead onto our current investigations: further exploration of the signalling mechanisms at the molecular level in COPD lungs. We hypothesise that a reduction in peroxisome proliferator-activated receptor (PPAR) activity in COPD patients mediates the unregulated inflammatory response present in COPD airways. PPARs are ligand-activated transcription factors belonging to the nuclear steroid hormone receptor superfamily [2]. Natural ligands, such as fatty acids and eicosanoids (polyunsaturated fatty acids, leukotriene B4), and synthetic ligands (glitazones and the fibrates) have been identified. The ligand–PPAR complex can transcriptionally regulate genes associated with lung inflammation [3, 4], such as the inducible form of cyclooxygenase (COX)-2, interleukin (IL)-6, IL-8 and tumour necrosis factor (TNF)-α, and antioxidant enzymes, such as catalase, Cu and Zn-superoxide dismutase and the redox enzymes of the glutathione pathway.

In vitro experiments with the alveolar epithelial cell line, A549, demonstrated the anti-inflammatory capacity of PPAR agonists. Utilising a lung tissue bank, we assessed PPARα protein levels in patients with COPD who were current or ex-smokers in comparison with nonsmokers and healthy smokers. Although not significant, increased PPARα levels were observed in lung tissue from healthy smokers and ex-smokers with COPD, but not for current smokers with COPD. Assessing the levels of PPARα mRNA showed an increase in all patient groups compared with nonsmokers, suggesting that a post-transcriptional or post-translational defect has resulted in reduced PPARα protein in the lungs of COPD patients.

MY RESEARCH AS PART OF MY WORKING GROUP/ RESEARCH TEAM

As inflammation is a central theme to the work of our group as well as the larger department, the Medical Research Council/ Centre for Inflammation Research, my research falls well within the remit of both.

Lung inflammation is a prominent feature of both COPD and particle inhalation. For both, the inflammatory response and resulting lung injury have been associated with oxidative stress as follows: 1) in COPD, either as a result of smoking or indirectly by the release of increased amounts of reactive oxygen species from airspace leukocytes; and 2) in particle exposure, due to particle size and composition-dependent free radical generation. An excess of oxidants may then lead to enhanced pro-inflammatory gene expression and protein release, and oxidative tissue injury. My studies assessing the molecular signalling mechanism relating to the regulation of inflammation in lung tissue have clear associations with COPD patient phenotyping and biomarker studies, as oxidative stress-initiated signal transduction results in the generation of pro-inflammatory mediators that are being investigated as potential biomarkers for COPD. Moreover, this work can be linked with the particle research, as cigarette smoking, the main aetiological factor in COPD, is also an inhaled toxicant comprising particulates. Our studies may also apply to other inflammatory conditions where oxidative stress plays a role.

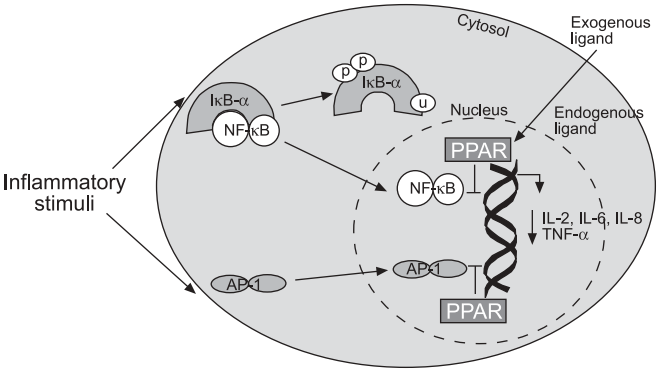

FIGURE 1. A schematic representation of the proposed action of proliferator-activated receptor (α- and γ-isoforms) in the inhibition of nuclear factor (NF)-κB and activator protein (AP)-1-mediated gene transcription. IκB-α: NF-κB-α inhibitor; IL: interleukin; TNF: tumour necrosis factor.

THE IMPACT OF MY WORK ON CLINICAL OR RESEARCH PRACTICE

We propose that PPAR may be a normal braking mechanism on inflammation in smokers and that this braking system is diminished in that proportion of smokers who develop COPD. We hypothesise that, without the constraints commonly imposed by PPAR activation, the inflammatory response in COPD airways is allowed to continue unhindered. By redressing this imbalance, we propose that PPAR agonists may be a potential anti-inflammatory therapy in COPD by targeting NF-κB and thereby NF-κB-dependent pro-inflammatory genes. Although this research is in the early stages, these studies will inform the design of clinical trials with PPAR agonists, a realistic aim since PPAR agonists are already available for use in inflammatory diseases and diabetes.

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