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COMMENTARY

Targeting airway inflammation: PMX464 and the epithelial bulls eye

DW Sexton

BioMedical Research Centre, School of Medicine, Health Policy and Practice, University of East Anglia, Norwich, UK

Increasing evidence places the epithelial cell at the centre of inflammatory processes in human airways. Crucial to this function and the maintenance of inflammatory homoeostasis is a balanced oxidant–antioxidant status in the airway, in part controlled by thioredoxin and thioredoxin reductase, which together can alter the NF-κB pathway. PMX464, a thiol-reactive quinol and putative thioredoxin inhibitor, has been investigated in endothelial cells, fibroblasts and colorectal cancer cell lines but in the present issue of the *BJP*, these investigations were extended to A549 airway epithelial cells. Thioredoxin inhibition was confirmed as was NF-κB and IKK suppression but siRNA knockdown of thioredoxin did not alter inflammatory marker expression or activity, suggesting that PMX464 has targets other than thioredoxin. Future consolidation of this evidence will involve concomitant knockdown of thioredoxin reductase, the use of primary airway epithelial cells and, potentially, the employment of three-dimensional (3D) culture systems for both A549 and primary cells.
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Abbreviations: CF, Cystic fibrosis; COPD, Chronic obstructive pulmonary disease; IKK, IκB Kinase

Epithelial surfaces represent the front line of immune defences and their innate immune capacity and interaction with the adaptive arm of immunity have placed these cells at the centre of inflammation initiation, maintenance and resolution (Hayday and Viney, 2000; Walsh *et al*., 2003). In many inflammatory diseases, the airway epithelium becomes damaged, which results in the idea that the epithelial cell layer is a relatively passive victim of inflammation. This paradigm has shifted, however, with increasing evidence highlighting the central function of the epithelium in the inflammatory processes of the airway, including in asthma, chronic obstructive pulmonary disease (COPD) and cystic fibrosis (CF) (Stick and Holt, 2003; Holgate, 2008).

A key factor in airway epithelial cell function and overall airway homoeostasis is the maintenance of the oxidant–antioxidant balance, which is significantly affected by the thioredoxin–thioredoxin reductase system. Aberrations are known to lead to a variety of airway diseases, such as asthma, COPD and idiopathic pulmonary fibrosis (Rahman *et al*., 2006). Indeed, we know that oxidative stress in asthmatic airways can lead to the activation of the redox-sensitive transcription factors, NF-κB and AP1, and that blockade of these transcription factors in a mouse model of asthma with the thioredoxin inhibitor, MOL 294, decreased bronchoalveolar lavage levels of eosinophils, IL-13, and eotaxin as well as diminishing airway tissue eosinophilia and mucus hypersecretion (Henderson *et al*., 2002).

Consequently, in this issue of the *BJP*, Callister *et al.,* (2008) have investigated the effects of the thiol-reactive quinol and thioredoxin inhibitor, PMX464, in the human type II alveolar adenocarcinoma cell line, A549. The paper expands the range of target cells for PMX464 to airway epithelial cells and, through assessment of several markers of inflammation, shows it to be a potent anti-inflammatory agent in this cell type.

The data presented clearly identifies suppression of NF-κB and its related kinase, IKK, as part of the anti-inflammatory mechanism and the authors also confirm thioredoxin inhibition in this cell type. Thioredoxin has been implicated in asthma pathogenesis; both in the airway hyperresponsiveness and the inflammation observed (Ichiki *et al*., 2005). Its pro-inflammatory or anti-inflammatory status depends on whether it is in its reduced or oxidized state, respectively; an important point when discussing this paper and the thioredoxin reductase activity in airway epithelial cell lines. Intriguingly, reduced thioredoxin also has the capacity to act as a mucolytic in CF patients, decreasing the viscosity of sputum samples. In the same study, Rancourt *et al.,* (2007) also analysed the IL-8 cytokine response of 16HBE cells (a differentiated, SV-40 transformed, bronchial epithelial cell line) to reduced thioredoxin. IL-8 expression was augmen-

Correspondence: Dr DW Sexton, BMRC, School of Medicine, Health Policy and Practice, University of East Anglia, Norwich NR4 7TJ, UK.
E-mail: d.sexton@uea.ac.uk
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ted, which hinted at the potential for cytokine modulation with a thioredoxin inhibitor, such as PMX464.

Although PMX464 was known to disrupt the thioredoxin/thioredoxin reductase redox pathway, the authors employed the ever-burgeoning method of siRNA to confirm the thioredoxin-specific effects of PMX464's actions. Experiments with siRNA against thioredoxin suggested that PMX464 could work through an, as yet, undetermined non-thioredoxin pathway. This is exciting, if true, but comes with some caveats. The authors did not knock down thioredoxin reductase expression, as this enzyme has not been implicated in earlier publications on PMX464, although they do disclose that they have preliminary data identifying such activity. Of equal import is the employment of the much-utilized A549 cell line. As conceded by the authors, although a well-studied cell line, A549 cells are derived from type II alveolar adenocarcinoma and, consequently, are altered in their phenotype, relative to normal human alveolar epithelial cells. Altered gene expression and biochemistry are ever-present concessions in research endeavours using cell lines. It is imperative, therefore, that the basic biochemistry in cell lines and their normal equivalents are understood. Appropriately, the authors cite Soini *et al*., (2001) who studied thioredoxin and thioredoxin reductase expression in several lung cell lines, including BEAS-2B, a normal, transformed cell line and A549 cells. Intriguingly, although thioredoxin was similar in all cell lines, thioredoxin reductase was significantly higher in A549; not surprising because perturbation of the thioredoxin system has been implicated in cancer survival with thioredoxin reductase now targeted for therapeutic development (Pennington *et al*., 2007).

As elevated expression of a component of a pathway under investigation can have a confounding effect on a study, Callister *et al.,* (2008) concede that they have further investigations to carry out. The authors plan to investigate primary airway epithelial cells, which better represent the airway epithelial cells *in vivo* but there is still the opportunity here for altered pathways. The reasoning behind this statement is the contrast between *in vivo* cell phenotype and the phenotype of *in vitro* cultured structural cells.

Drug screening and research using airway epithelial cells always need to consider the assessment of primary cells in various growth conditions, such as three-dimensional (3D), rather than monolayer, culture systems and with air–liquid interfaces. *In vivo,* cells are not only in contact with other cells but also with the extracellular matrix (ECM) that forms a scaffold for their growth and organizes communication among those cells within the matrix. Weaver *et al*., (1997) found that the structural cells grown in monolayers and 3D culture systems responded differently to drugs. Although not necessarily the case with the thioredoxin pathway in airway epithelial cells, it is worth considering for future investigations. An understanding of the differences in the gene expression and the phenotype of these cells may yield outcomes salient to drug assessment *in vivo*. Indeed, the ramifications extend to 'failed' drugs, which may have been inappropriately 'shelved' during preliminary *in vitro* screening but may now be subject to re-evaluation in 3D culture systems.

Cost, availability and ease of manipulation are often factors determining initial experimental design and it is understandable, therefore, that cell lines are much-utilized in the early stages of investigations. As a continuation of this study, the authors may wish to explore the growth of A549 cells in such 3D cultures with an air–liquid interface. As outlined above, such conditions can alter phenotypes and may return the A549 cells to a level of thioredoxin reductase activity, more comparable with non-cancerous airway epithelial cells. Validation of the A549 cells in such culture systems for probing of the thioredoxin system would provide a valuable addition to this research area as primary small airway epithelial cells are expensive to obtain or purchase and subsequently culture.

The extent to which PMX464 can target other cell types is important to its value as a therapeutic candidate. PMX464 seems to have pleiotropic effects on multiple cell types, including fibroblasts, human colorectal epithelial cells, human endothelial cells and now human airway epithelial cells. For airway inflammation research, the effect of PMX464 on eosinophil and neutrophil function is an important avenue of investigation. For airway epithelial cells, the effect of PMX464 on chemokine and cytokine profiles, lipid mediator release, mucin expression and on the rheology of mucus derived from airway diseases other than CF, are fascinating future goals, as are the identification of other protein targets for PMX464 activity. Callister *et al.,* (2008) have taken a positive step forward in characterizing this drug's potency and mode of action in this important target cell type.

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