## P49 THE EFFECTS OF ORAL COTRIMOXAZOLE UPON NEUTROPHIL AND MONOCYTE ACTIVATION IN PATIENTS WITH PULMONARY FIBROSIS AND HEALTHY CONTROLS; DOES THIS RELATE TO ITS ACTION IN IDIOPATHIC PULMONARY FIBROSIS?

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Sulfamethoxazole and dapsone share the same sulphonamide ring with similar antibacterial effects. Detailed studies of dapsone show extensive effects on the immune system with the reduced generation of oxygen free radicals (ROS) and inhibition of neutrophil (NΦ) myeloperoxidase. These effects reduce intra and extracellular ROS reducing endothelial damage, lipid peroxidation and apoptosis. The bacterial peptide N-formyl-met-leu-phe (fMLP) activates the NΦ via its formyl peptide receptor (FPR) generating ROS much of which is extracellular. The FPR receptor also induces cell migration, granule secretion and lysosomal activation. Dapsone very significantly reduces fMLP activation of NΦ, giving anti-inflammatory effects. Interestingly phorbol 12-myristate 13-acetate (PMA), a well characterised activator of protein kinase C is less inhibited by dapsone. There are no comparable studies with cotrimoxazole despite its structural similarity. UK clinical studies of cotrimoxazole in Idiopathic pulmonary fibrosis (IPF), suggest protection against sudden severe exacerbations. Cotrimoxazole is thought to act via its anti-bacterial properties with data showing pathogen carriage in a third of new IPF cases. Oxidative stress is increased in IPF and limited studies show that peripheral monocyte (MΦ) depletion via a charcoal column in IPF exacerbations has reduced the 30 day mortality. In fibrotic organ injury, recruitment of MΦ appears critical and blocking MΦ activation and recruitment arrests the fibrotic process. We have examined by flow cytometry the effects of oral cotrimoxazole upon NΦ and MΦ activation in IPF patients on long-term treatment and healthy controls at baseline and after 1 week of cotrimoxazole. The commercial kit PHAGOBURST (Glycotope Biotechnology) was used, which allows quantitative determination of leucocyte oxidative burst in whole blood following stimulation by opsonized E Coli, PMA and fMLP.

**Findings** Despite the small numbers to date, similar to dapsone there is a significant blocking of oxidative burst to fMLP in NΦ and MΦ pre- and post cotrimoxazole with a similar trend in IPF. There is also some reduction in PMA oxidative burst, but none to E.coli. The reduced MΦ stimulation may reflect the lower MΦ bloods counts. If NΦ and MΦ ROS generation are reduced by cotrimoxazole, this may stabilise the disease process protecting against severe exacerbations.

## P50 LOCALISATION OF THE GLYCOLYTIC ISOZYME, PYRUVATE KINASE M2 IN THE LUNG OF IDIOPATHIC PULMONARY FIBROSIS

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**Introduction** Idiopathic pulmonary fibrosis (IPF) is the most common form of interstitial lung disease, with a poor prognosis and a lack of therapeutic options that halt disease progression. While the aetiology of IPF is unknown, dysregulated epithelial and mesenchymal response following persistent epithelial insult is thought to be critical in driving fibrosis. Recent evidence suggests that, akin to cancer, metabolic reprogramming may be important in driving many of these processes. In both cancer and fibrosis development, inducible expression of the pyruvate kinase isoform M2 (PKM2) represents an important adaptation for increasing the availability of glycolytic intermediates for biosynthesis and cell proliferation.

**Aim** We aim to investigate the expression of PKM2 in relation to cell-specific markers in the IPF lung.

**Abstract P49 Table 1**

| **Neutrophils** **Mean fluorescence*±SEM of stimulated cells (arbitrary units)** | **Healthy controls (HC) n=9 No treatments** | **Healthy controls n=6 post-Cotrimoxazole 7 days (960 mgBD)** | **Paired t test +significance at the 5% level (pre- and post Cotrimoxazole n=6)** | **IPF on long-term Cotrimoxazole (mean treatment duration 23 months, Cotrimoxazole (960 mgBD) n=8** | **Mann Whitney U test HC versus IPF +significance at the 5% level** |
|---|---|---|---|---|---|
| Mean neutrophil blood count $10^9$/l (SEM) | 3.32±0.24 | 3.43±0.16 | | 4.49±0.39 | |
| PMA Nϕ | 7014±1623 | 5903±1503 | p=0.683 | 3866±823 | p=0.117 |
| fMLP Nϕ | 989±242 | 246±244 | **p=0.015**+ | 448±407 | p=0.08 |
| E coli Nϕ | 4417±447 | 3302±815 | p=0.331 | 3606±358 | p=0.189 |
| **Monocytes** **Mean fluorescence*±SEM of stimulated cells (arbitrary units)** | **Healthy controls (HC) No treatments n=9** | **Healthy controls n=6 Cotrimoxazole 7 days (960 mgBD)** | **Paired t test +significance at the 5% level (pre- and post Cotrimoxazole n=6)** | **IPF on long-term Cotrimoxazole (mean treatment duration 23 months Cotrimoxazole (960 mgBD) n=8** | **Mann Whitney U test HC versus IPF +significance at the 5% level** |
| Mean monocyte blood count $10^9$/l (SEM) | 0.55±0.06 | 0.53±0.08 | | 0.77±0.07 | |
| PMA Mϕ | 503±149 | 236±109 | p=0.99 | 424±166 | p=0.541 |
| fMLP Mϕ | 1467±460 | 38±24 | **p=0.05**+ | 1340±807 | p=0.276 |
| E coli Mϕ | 990±168 | 787±136 | p=0.57 | 808±144 | p=0.733 |

*fluorescine agent dihydrorhodamine123-maximum absorption 488–490 nm.