Multidrug Resistance-Associated Protein 1 (MRP1) Expression and Activity in Human Distal Lung Epithelial Cells

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Purpose
MRP1 has been reported to have the highest protein abundance of all ABC transporters studied in human lung tissues (1). The subcellular localisation and functional activity of the transporter in distal lung epithelium, however, remains poorly understood. The aim of this study was to determine MRP1 expression levels, localisation and activity in the NCI-H441 cell line, and compare those data with freshly isolated human alveolar type (AT) II and type I-like epithelial cells in primary culture.

Methods
MRP1 expression in ATII and ATI-like cells from three different patients as well as in at least three different passages of NCI-H441 cells were studied using q-PCR and immunoblot. Transporter localisation was confirmed by confocal laser scanning microscopy (CLSM) and cell surface biotinylation studies. Efflux of the MRP1 substrate 5(6)-carboxyfluorescein (CF) (converted intracellularly from its diacetate conjugate, CFDA) from NCI-H441 cells was measured in the presence and absence of a series of known MRP1 modulators (i.e. MK-571, verapamil, indomethacin and quinidine) as well as common inhaled drugs (i.e. albuterol, formoterol, budesonide and beclomethasone). Bidirectional transport studies of CFDA across monolayers of ATII-like and NCI-H441 cells were also carried out to assess transporter activity.

Results
MRP1 was found to be stably expressed in NCI-H441 cells across passage numbers 59-81. Expression levels in NCI-H441 cells were also unaffected when the cells were cultured at an air-liquid interface. MRP1 expression intensity in primary cells did not change during transdifferentiation from an ATII to an ATI-like phenotype. CLSM gave inconclusive results with regards to MRP1 localisation, but cell surface biotinylation followed by immunoblot confirmed the transporter in basolateral cell membranes. Efflux and transport studies in were consistent with expression data. Efflux of CF was inhibited by MK-571 (>90% inhibition at 50 μM), verapamil (20% inhibition at 50 μM), indomethacin (30% inhibition at 10 μM) and quinidine (25% inhibition at 100 μM). Both β-agonists increased CF efflux significantly. Albuterol (200 μM) by 60% and formoterol (100 μM) by 20%. The inhaled corticosteroids (both at 50 μM) reduced CF efflux; budesonide by 20% and beclomethasone by 30%. Interestingly, when budesonide (5 and 10 μM) was added to the culture medium of NCI-H441 cells for up to 72 h prior to start of the efflux experiments, CF retention was significantly reduced in a time and concentration-dependent manner. Bidirectional transport studies across monolayers of ATII-like and NCI-H441 cells showed significant net absorption, which was sensitive to inhibition with MK-571. Absorptive \( P_{app} \) values (all x10⁻⁵ cm/s) in ATII-like cells were 1.02±0.03 (A-to-B), 0.34±0.05 (B-to-A), 0.71±0.03 (A-to-B MK-571), 0.60±0.15 (B-to-A MK-571), and in NCI-H441 cells 2.37±0.12 (A-to-B), 1.67±0.12 (B-to-A), 0.97±0.09 (A-to-B MK-571), 1.30±0.13 (B-to-A MK-571), respectively.

Conclusion
MRP1 was confirmed to be expressed at similar levels and activities in freshly isolated human alveolar epithelial cells in primary culture and in the NCI-H441 cell line. It can thus be suggested that NCI-H441 cells can be used as an in vitro model for the study of MRP1 transport in distal lung epithelial barrier. Commonly prescribed inhaled medicines had significant effects on MRP1 functionality, which might be of clinical relevance, as MRP1 variants have previously been linked with lung function and inflammatory markers in COPD patients (2).

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