Role of multidrug resistance-associated protein 1 in airway epithelium

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CHAPTER FOUR

Multidrug resistance-associated protein 1 in transplant and native bronchus epithelium of emphysema patients

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Abstract

Objective: Smoking is the principal risk factor for development of chronic obstructive pulmonary disease (COPD). Multidrug resistance-associated protein 1 (MRP1) is cytoprotective against oxidative stress and xenobiotics and is highly expressed in normal bronchial epithelium. We questioned whether MRP1 expression is lower in epithelium of COPD lung compared to healthy donor lung.
Methods: Primary bronchial epithelial cells were isolated from airway wall brushes of six patients with severe COPD who underwent lung transplantation. Two brushes were obtained of every patient, one from the donor and one from the native part of the bronchus. MRP1 mRNA was measured by quantitative RT-PCR (n=4), MRP1 mediated activity by functional FACS analysis (n=6).
Results: MRP1 mRNA levels were higher in epithelial cells of the donor than native COPD bronchus ($p=0.023$). In four out of six cases, MRP1 activity was higher in epithelial cells from the donor bronchus. MRP1 expression in the donor and the native epithelial brushes correlated well for both mRNA levels and MRP1 activity ($r=0.97$, $p=0.027$ and $r=0.94$, $p=0.005$ respectively).
Conclusion: MRP1 expression is higher in bronchial epithelial cells of healthy donor bronchus compared to the native COPD bronchus after lung transplantation. Further studies are required whether MRP1 plays a protective role against development of COPD.

Introduction

Smoking generates oxidative stress in the lung and is the principal risk factor for development of chronic obstructive pulmonary disease (COPD). Proteins of the ATP-binding cassette (ABC) superfamily such as the multidrug resistance-associated protein 1 (MRP1) protect against oxidative stress and xenobiotics [1]. Especially MRP1 is extensively expressed in human lung, mainly at the basolateral side of bronchial epithelial cells [2-4]. We observed that MRP1 expression is lower in bronchial epithelial cells of COPD patients compared to healthy ex-smokers [5]. In the present pilot study, we questioned whether MRP1 transcriptional expression levels and functional MRP1 mediated transport differs between bronchial epithelial cells in the airways of healthy donor lung and severe COPD. We analyzed airway wall brushes of patients with severe COPD following lung transplantation. This setting offers the opportunity to compare epithelial cells of the airway wall in a healthy donor lung and a lung of a COPD patient in the same individual, independent of external factors such as present use of medication that might otherwise influence MRP1 functional expression.

Patients and methods

Six lung transplant recipients with severe COPD were included (for characteristics see Table 1). Bronchoscopy was performed as scheduled for routine reasons, i.e. follow-up after lung transplantation or on clinical indication. Primary bronchial epithelial cells were collected from two brushes, one proximal and one distal to the suture between transplant and donor tissue. Cells were cultured for two passages in bronchial epithelial growth medium (BEGM) (Cambrex Corporation, The Netherlands) and grown on tissue culture plastics coated with Vitrogen (Nutacon, The Netherlands), fibronectin (Sigma, The Netherlands) and bovine serum albumin (Merck, The Netherlands). The protocol was approved by the Medical Ethics Committee and all patients gave informed consent. All patients quitted smoking at least 2 years before lung transplantation and received as immunosuppressants prednisolone and tacrolimus after lung transplantation; 4 patients (# 1, 2, 4 and 5) were treated with azathioprine. Three out of six COPD patients had a proven mutation in the alpha1-antitrypsin (AAT) gene, the others were negative for mutation in this gene.

Table 1. Characteristics of patients with severe COPD who underwent lung transplantation.

Patient	Diagnosis	Age	Packyears	Analysis
1	COPD	54	22	qPCR/FACS
2	COPD	55	20	qPCR/FACS
3	COPD (AAT)	47	3	qPCR/FACS
4	COPD (AAT)	59	5	qPCR/FACS
5	COPD	56	5	FACS
6	COPD (AAT)	58	13	FACS

Abbreviations: COPD, chronic obstructive pulmonary disease; AAT, alpha1-antitrypsin deficiency; qPCR, quantitative polymerase chain reaction; FACS, fluorescent activated cell sorter.

Total RNA was isolated and analysed for MRP1 mRNA expression with quantitative reverse transcriptase polymerase chain reaction (RT-PCR). Glyceraldehyde-3-phosphate dehydrogenase (GADPH) mRNA served as internal control. To determine MRP1 mediated transport, 1 x 10^6 cells were incubated with 0.1 μM carboxyfluorescein diacetate (CFDA, which is converted to CF, a fluorescent MRP1 substrate) as described previously [6]. After 2 hours incubation, fluorescence of CF was analysed with a FACSCalibur™ flow cytometer to determine the mean fluorescence intensity (MFI).

Differences between MRP1 activity and expression between donor and native bronchial cells were tested with the paired Student's t-test, correlations were tested with the Pearson test. Statistical analyses were performed with SPSS 10 (SPSS Inc., Chicago, IL).

Results

Relative MRP1 mRNA levels were higher in bronchial epithelial cells of the donor than of the native airway in 4 evaluable subjects (p=0.023) (Figure 1). One patient could not be analysed because of insufficient cell recovery, the other due to poor RNA quality of the donor location of the bronchus (Table 1). MRP1 mediated activity was higher in 4 out of 6 brushes from the donor location compared to the native part. This difference was not significant in the whole group (p=0.186) (Figure 2). In one patient, CF accumulation was the same in donor and native bronchial epithelial cells and in one patient CF

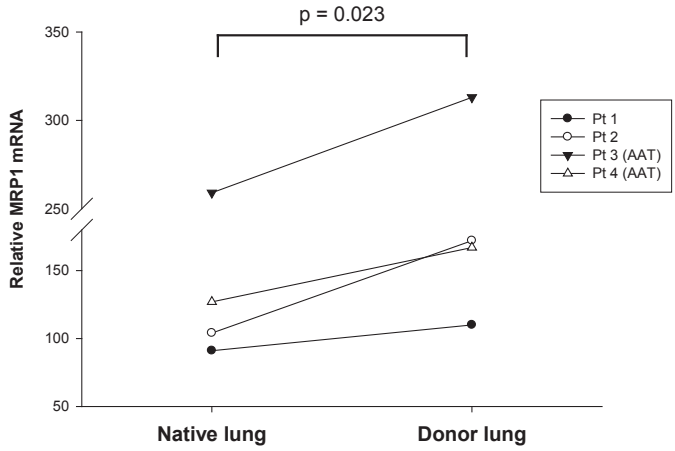

Figure 1. MRP1 mRNA levels (relative to GAPDH values) in primary bronchial epithelial cells from brushes of the native and donor part of the bronchus of four patients transplanted because of severe COPD. Pt: patient; AAT: alpha1-antitrypsin deficiency.

accumulation was higher in donor than in native bronchial epithelial cells. There was a strong correlation between MRP1 mRNA levels in epithelial cells from brushes of native and donor bronchi (Pearson r=0.97, p=0.027). Also, MRP1 mediated activity of epithelial cells in brushes of the native bronchi correlated significantly with that of the donor bronchi (r=0.94, p=0.005).

Discussion

These results demonstrate for the first time a higher MRP1 level in transplanted "healthy" bronchial epithelial cells compared to the native epithelial cells from patients with severe COPD, yet derived from the same lung transplant recipient. Thus our results support the hypothesis that lower expression or function of MRP1 is related to COPD [2, 5]. This new finding is worth to explore in larger patient studies and in other lung diseases as well. Factors that may otherwise have a substantial influence on MRP1 activity e.g. current smoking, air pollutants, and drug treatments [7, 8] can be excluded as confounders in our study, since they have most likely similar effects in brushes of the same individual. Though interindividual differences exist, both the MRP1 mRNA levels and activities correlated well between donor and native brushes of the same patients. This suggests that

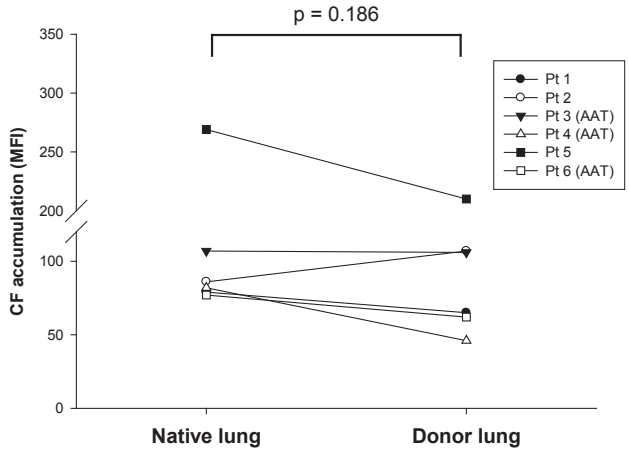

Figure 2. MRP1 activity in primary bronchial epithelial cells from brushes of the native and donor part of the bronchus of six patients transplanted because of severe COPD. Pt: patient; AAT: alpha1-antitrypsin deficiency; CF: carboxyfluorescein; MFI: mean fluorescence intensity.

external factors, e.g. smoke exposure or use of medication, may indeed have influenced these interindividual differences.

Three out of six COPD patients had a mutation in the AAT gene, which is strongly related to emphysema. There is no information available about a possible relationship between MRP1 and AAT. The frequency of the most common mutation of AAT, the Z-allele, occurs in 95% of all cases with AAT-related emphysema. The consequence is that AAT cannot be excreted and accumulates in hepatocytes and in bronchial cells [9]. It can be speculated that this has a direct or indirect influence on MRP1 expression or function by e.g. altered pH or higher expression of proteolytic enzymes (like e.g. mannosidase I) that cleave glycosylated proteins such as AAT and possibly MRP1 as well. Thus, the deficiency of AAT may affect MRP1 functional activity and this may contribute to the severity of emphysema.

In conclusion, MRP1 levels are higher in bronchial epithelial cells of healthy donor bronchus compared to the native part of the bronchus in patients with severe COPD who underwent lung transplantation. Further studies are required to confirm these results in larger patient groups and especially to explore whether MRP1 plays a protective role against development of COPD by transporting toxic compounds generated by smoke.

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