Role of multidrug resistance-associated protein 1 in airway epithelium

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CHAPTER ONE

General Introduction

General introduction

Chronic obstructive pulmonary disease (COPD) is characterized by extensive airway obstruction and chronic inflammation. Cigarette smoking is the most important risk factor for COPD. More than 90% of all COPD patients are smokers or ex-smokers, but only 15 to 20% of all smokers develop COPD [1]. Smoking is known to generate oxidative stress in the lung and this has led to the hypothesis that an imbalance between oxidants and anti-oxidants underlies the development of COPD [2]. Several mutations in the alpha-1-antitrypsin gene, which encodes an anti-proteinase protein, have been shown to contribute to COPD development. This suggests that an imbalance of proteinases and anti-proteinases plays a role in the pathogenesis of COPD as well. However, it was found that only a minority (1% to 3%) of COPD patients has mutations in this gene [3], thus other genetic or environmental factors were expected to play a role.

Little is known about detoxification mechanisms in the lung upon inhalation of cigarette smoke. Glutathione (GSH) is an important anti-oxidant present in every cell of the human body with very high levels in the lung. GSH levels are increased in epithelial lining fluid of lungs of chronic smokers whereas these levels are depleted during active smoking [4]. In addition, it has been shown that GSH levels are increased in lungs of COPD patients (current smokers). This is thought to be an attempt to resist the oxidative stress inducing effects of cigarette smoke rather than reflecting disease severity. The GSH levels may have been directly influenced by recent smoking.

Protein members of the ATP-binding cassette (ABC) superfamily of transporters are able to reduce oxidative stress. These transmembrane proteins can transport a wide variety of compounds across biological membranes in an ATP-dependent manner. In 1983, overexpression of the ABC transporter P-glycoprotein (P-gp) in tumor cells was discovered to cause resistance to several chemotherapeutic drugs with unrelated structures, resulting in so-called multidrug resistance (MDR) [5]. This was followed by the discovery of multidrug resistance-associated protein 1 (MRP1) in 1992 in a small cell lung cancer cell line [6, 7]. Many cytostatic agents are substrates for MDR proteins and as a consequence, overexpression of these transporters can contribute to failure of chemotherapy. At present, there are 48 human ABC transporters known, divided in seven subfamilies denoted as ABCA to ABCG [8, 9]. MRPs (MRP1 to MRP9) are members of the ABCC subfamily of

ABC transporters. Besides MRP1, also MRP2, 3, 4, 5, 7, and 8 have been to described to confer MDR [10-15]. MRP1 is a membrane transport protein that is highly expressed in the human lung, especially in the bronchial epithelium [16-18]. However, its function in the lung is as yet unknown.

Substrates for MRP1 (and the other MRPs) are GSH-, glucuronide-, and sulfate-conjugates, oxidized GSH (GSSG), or GSH together with unconjugated substrates [10, 19, 20]. Cellular GSH levels highly depend on MRP1 functional activity and thus, MRP1 plays a central role in the protection against oxidative stress (Figure 1).

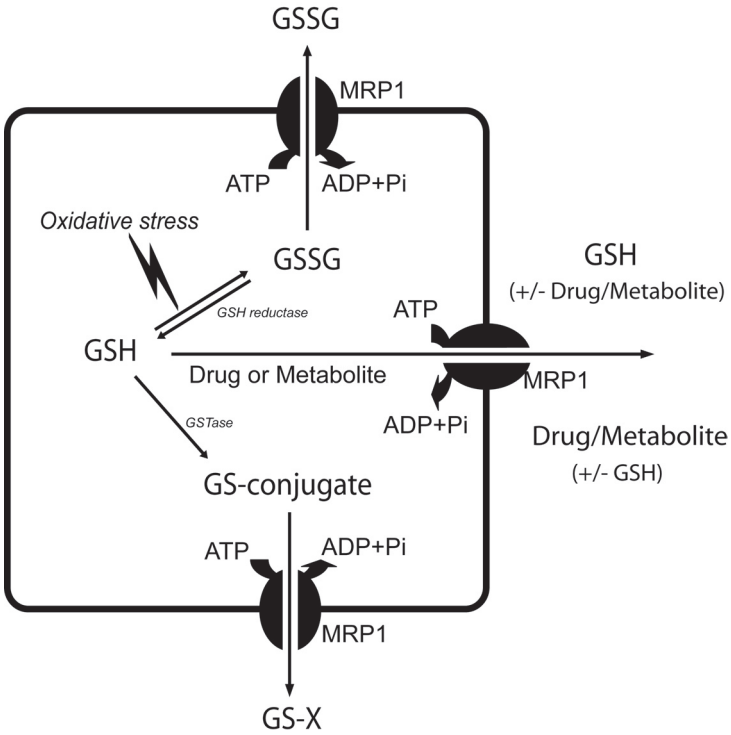

Figure 1. Detoxification pathways in which MRP1 and GSH play a central role.
GSH, glutathione; GSSG, oxidized glutathione; GS-X, GS-conjugate; ATP, adenosine triphosphate; ADP, adenosine diphosphate; Pi, phosphate.
Figure adapted from: Leslie *et al.*, Toxicology 2001:167:3-23.

Aim and outline of the thesis

The notions that MRP1 is protective against oxidative stress and is highly expressed in the human lung has led to the hypothesis that MRP1 might be important to protect against inhaled toxic substances such as present in cigarette smoke. In this thesis we postulate that MRP1 expression or activity is less functional in lungs of individuals who are prone to develop COPD. Insight in functional mechanisms of MRP1, potentially involved in protection against cigarette smoke-induced toxicity, may open new possibilities for better treatment of pulmonary diseases such as COPD. In this thesis we investigated whether MRP1 levels in bronchi and lung tissue are related to COPD development. Preclinical studies as well as studies in patient samples were performed.

In **chapter two**, a literature review of ABC-transporters in normal and pathological lung is given. This review is focused on polymorphisms, knockout mice models and *in vitro* results of pulmonary research.

To investigate the possibility that MRP1 or other MDR proteins are differentially expressed in lungs of COPD patients, we describe in **chapter three** the analysis of MRP1, P-gp and lung resistance-related protein (LRP) expression levels in bronchial biopsies and lung tissue of COPD patients and healthy controls by means of immunohistochemistry. To investigate whether these proteins are affected by cigarette smoke exposure and whether expression is related to the rate of lung function decline, the correlation between their expression levels with smoking history (packyears) and lung function parameters was analyzed.

In **chapter four**, we analyzed MRP1 activity and transcription levels in primary epithelial cells. These cells were obtained from lung brushes of the native ("COPD") and donor ("healthy") parts of lungs of emphysema patients after lung transplantation.

In **chapter five,** the *in vivo* role of MRP1 and P-gp was studied in smoke-induced lung pathology and inflammation. This chapter describes the results of cigarette smoke exposure in triple knockout mice that lack the genes for Mrp1 and the two murine genes for P-gp (*Mdr1a* and *Mdr1b*). These mice were chosen because it is known that the transporters MRP1 and P-gp are highly expressed in the lung. It was analyzed whether these knockout mice were more at risk than wildtype mice to develop emphysema after six months smoke exposure. In addition, differences in pulmonary inflammation were determined between the knockout and wildtype mice, as this inflammation represents in general a main pathogenetic factor in the

development of COPD/emphysema.

To study the effects of tobacco smoke on MRP1 activity and cell survival, we incubated an immortalized bronchial epithelial cell line with cigarette smoke extract (CSE). Results are described in **chapter six**. Cell survival was measured with a cytotoxicity test and effects on MRP1 activity were determined with functional flow cytometry by using a fluorescent MRP1 substrate. MRP1 was downregulated with RNA interference to show the MRP1 specificity of the effects of CSE.

In **chapter seven**, we aimed to gain more insight in the possible effects of COPD treatments on MRP1**.** MRP1 activity was modulated with several pulmonary drugs in bronchial epithelial cells by means of flow cytometry. For this purpose, cells were incubated with budesonide (a corticosteroid), formoterol (a long-acting beta-agonist), or their combination, N-acetylcysteine (an antimucolytic agent with antioxidant properties) and ipratropium bromide (an anticholinergic drug).

In **chapter eight**, we describe the effects of the non-steroidal anti-inflammatory drug (NSAID) and MRP1 inhibitor indomethacin on MRP1 in a small cell lung cancer cell line. The purpose of this study was to determine a relation between MRP1 expression in lung tumor cells and sensitivity for indomethacin. Effects of indomethacin on cell survival, apoptosis, MRP1 mediated activity, GSH levels and mitochondrial potential were analyzed in lung tumor cells with high and low MRP1 levels.

In **chapter nine**, a summary is given of this thesis together with the future perspectives for further research, followed by a summary in Dutch.

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