Molecular and Functional Expression of Multidrug Resistance-Associated Protein-1 in Primary Cultured Rat Alveolar Epithelial Cells

LEENA N. PATEL,[1] TOMOMI UCHIYAMA,[1] KWANG-JIN KIM,[2,3] ZEA BOROK,[2,3] EDWARD D. CRANDALL,[2,3] WEI-CHIANG SHEN,[1] VINCENT H.L. LEE[1]

[1]Department of Pharmacology and Pharmaceutical Sciences, University of Southern California, Los Angeles, California 90089-9121

[2]Department of Medicine, University of Southern California, Los Angeles, California 90089-9121

[3]Will Rogers Institute Pulmonary Research Center, University of Southern California, Los Angeles, California 90089-9121

Received 14 March 2007; revised 1 June 2007; accepted 13 June 2007

Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/jps.21134

ABSTRACT: Multidrug resistance-associated protein-1 (MRP1) is an integral membrane efflux protein that is implicated in multidrug resistance in cancer, but it is also expressed in normal tissues. The objective of this study was to determine the expression, localization and functional activity of MRP1 in primary cultured rat alveolar epithelial cells of types I- and II cell-like phenotypes. RT-PCR data showed 550-base pair fragments in both types I- and II-like pneumocytes that exhibited 99% identity to the rat MRP1 isoform. Significant levels of MRP1 protein were detected by western analysis of immunoprecipitates in both cell types, and immunofluorescence combined with confocal laser scanning microscopy indicated basolateral localization of MRP1. Indomethacin (0–100 μM) increased fluorescein basolateral-to-apical transport, and accumulation of fluorescein in the cells, in a dose-dependent manner. We therefore conclude that the MRP1 gene is present in primary cultured rat epithelial cells of both types I- and II-like phenotypes and its corresponding protein (MRP1) is localized in the basolateral membrane of these cells. Primary cultured monolayers of rat type II-like pneumocytes appear to be a useful tool for screening MRP1 substrates designed for pulmonary delivery/targeting. J Pharm Sci 97:2340–2349, 2008

Keywords: MRP1; efflux pumps; cell culture; ABC transporters; pulmonary; multidrug resistance-associated proteins

INTRODUCTION

The pulmonary route is an attractive alternative for systemic delivery of protein/peptide drugs such as insulin, human growth hormone, and vasopressin due to their high bioavailability via this route.[1] Small molecule drugs such as cromolyn, morphine, 9-tetrahydrocanabinnol, and rizatriptan also show significant systemic absorption when administered by inhalation and in some cases are transported by active mechanisms.[2]

Tomomi Uchiyama's present address is First Department of Biochemistry, School of Pharmacy, Kyushu University of Health and Welfare, Nobeoka, Japan.

Vincent H.L. Lee's present address is Choh-Ming Li Basic Medical Science Building, Suite 626E. School of Pharmacy, The Chinese University of Hong Kong, Shatin, NT, Hong Kong.

Correspondence to: Vincent H.L. Lee (Telephone: 852-2609-6862; Fax: 852-2603-5295; E-mail: vincent.lee@cuhk.edu.hk)

Journal of Pharmaceutical Sciences, Vol. 97, 2340–2349 (2008)

Inhaled glucocorticosteroids like fluticasone propionate and budesonide, although intended for local pharmacological effect, exhibit complete systemic absorption after prolonged retention in the lungs.[2–4] In particular, the lower respiratory tract that consists primarily of the alveoli lined by alveolar epithelium (types I and II pneumocytes) serves as the major systemic absorption site because it provides an expansive surface area (70–140 m^2 in adult human lungs), has extensive vascularization and forms a relatively thin barrier to the systemic absorption of therapeutic agents. In contrast, the conducting airways of bronchi and trachea are lined by much taller epithelial cells, have comparatively a very small surface area, and receive a small fraction of lung blood flow. The conducting airways also exhibit mucociliary clearance activities for deposited exogenous materials.[5]

In order to exploit the lower respiratory tract for drug delivery purposes, it is crucial to understand the properties of the alveolar epithelium that serve as the rate-limiting barrier to systemic absorption. *In vitro* cell culture models have been utilized to study the barrier properties of pulmonary epithelia in recent years. For instance, Calu-3 (a human lung cancer bronchial epithelial cell line) serves as a useful cell culture model for the upper airway due to its ability to form well differentiated, polarized, and electrically resistant cell monolayers.[6,7] However, such a reliable cell line for the lower respiratory tract is not yet available. Instead, Cheek et al.[8] have established a primary culture model that exhibits phenotypic and morphologic characteristics of alveolar epithelium *in vivo*. The most crucial characteristic exhibited is the high transepithelial resistance (TEER, $>2000\ \Omega\,cm^2$) with well-formed tight junctions. It has been demonstrated that freshly isolated type II cells can transdifferentiate *in vitro* into type I cell-like pneumocytes over time.[9] The type I-like cells begin to express markers specific to type I alveolar epithelial cells with the concurrent loss of lamellar bodies and other hallmarks of type II cells. On the other hand, when freshly isolated rat type II cells are exposed to keratinocyte growth factor (KGF, 10 ng/mL), they do not transdifferentiate into type I cells but instead retain their type II cell phenotypic characteristics.[10] This primary rat alveolar epithelial culture model consisting of types I or II cell-like pneumocytes has been extensively utilized for over two decades for mechanistic studies of peptide[11,12] and protein transport across the alveolar epithelial barrier.[13,14] In addition, the expression and function of various transporters, receptors, and ion channels have been well studied.[15–17]

Since the cloning of the multidrug resistance-associated protein-1 (MRP1) gene by Cole et al.,[18] nine human MRP isoforms that show 30–56% homology have been reported.[19] cDNAs for rat homologues of MRP1-6 have been cloned and characterized, wherein they show 66–94% homology to human isoforms. MRP1 is a 190 kDa membrane protein that belongs to the ATP-binding cassette (ABC) transporter superfamily and is known to cause cellular efflux of a wide variety of xenobiotics. Like the efflux pump P-glycoprotein (P-gp), MRP1 transports a structurally and pharmacologically diverse set of substrates. Apart from the overlap of certain oncolytic agents such as vincristine, daunorubicin, and doxorubicin, MRP1 substrates are organic anions and conjugates of glutathione (GSH), glucuronide or sulfates (products of phase II metabolism),[20] whereas P-gp extrudes lipophilic, amphiphilic, and cationic substrates. To study the functional characteristics of MRP1, specific substrates such as leukotriene C,[21] fluorescein dye,[22,23] and 17β-estradiol[24] have been utilized for efflux measurements in the presence and absence of MRP1-specific inhibitors such as indomethacin, probenecid, and MK571.[22,25] In contrast to the apical localization of P-gp, MRP1 is localized primarily on the basolateral side of polarized cells.

The putative role of MRP1 in lungs is that of protection against xenobiotics and endobiotics, but it is also likely to have other physiological functions such as regulating the redox state in the cells by cotransporting MRP1 substrates with glutathione or as glutathione conjugates. In addition, it has been demonstrated that pathological lung conditions such as chronic obstructive pulmonary disease (COPD) or antiasthma drugs like budesonide, modulate MRP1 expression.[26,27] It is therefore imperative to study the MRP1 expression and functional relevance in the lungs. In the respiratory tract, basolateral expression of MRP1 has been shown in bronchial cells,[25,28,29] whereas granular cytoplasmic staining was observed in human nasal respiratory epithelium.[30] Expression of MRP1 has also been shown in the seromucinous glands and alveolar macrophages.[29] However, a definitive investigation of MRP1 expression in the alveolar epithelium is lacking.

The purpose of this study was to investigate the expression of MRP1 in the primary cultured rat