Journal of Pharmaceutical and Biomedical Analysis 154 (2018) 150–157

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Quantification of membrane transporter proteins in human lung and immortalized cell lines using targeted quantitative proteomic analysis by isotope dilution nanoLC–MS/MS

John K. Fallon [a], Nicole Houvig [b], Catherine L. Booth-Genthe [b], Philip C. Smith [a,*]

[a] Division of Pharmacoengineering and Molecular Pharmaceutics, Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC, 27599, USA
[b] Drug Metabolism and Pharmacokinetics, Respiratory Therapeutic Area, GlaxoSmithKline, King of Prussia, PA, 19406, USA

ARTICLE INFO

Article history:
Received 25 September 2017
Received in revised form 16 February 2018
Accepted 20 February 2018
Available online 22 February 2018

Keywords:
Cells
Proteomics
Quantitative
Targeted
Transporters

ABSTRACT

Information is needed on the expression of transporters in lung to inform drug development and therapeutic decisions. Much of the information currently available is from semiquantitative gene expression or immunometric densitometry studies reported in the literature. NanoLC–MS/MS (MRM mode) isotope dilution targeted quantitative proteomics was used here to quantify twelve selected transporters in fresh human lung membrane fraction samples and in the membrane fraction of selected immortalized human lung epithelial cell line samples. Fractionation was undertaken by homogenization in crude membrane lysis buffer followed by differential centrifugation of the homogenate. In lung membranes we found OATPs to be the most highly expressed transporters of those measured, followed by PEPT2 and ABCs (P-gp & BCRP). SLC22A transporters (OCTs 2 & 3 and OCTN1) were also found to be expressed. OATP2A1, also known as the prostaglandin transporter, was the most highly expressed transporter, being low in two subjects who were at least occasional smokers. One subject, a non-smoker, had an OATP2A1 concentration that was 8.4 times higher than the next nearest concentration, which itself was higher than the concentration of any other transporter. OATP2A1 is known, from gene expression and animal functional studies, to be present in lung. These results inform the understanding of xenobiotic disposition in the lung and show the distinct profile of transporters in lung compared to other tissues.

1. Introduction

The lungs are a site of action for many drugs [1] and a well established route of drug administration [2]. Preclinical data for certain transporters is now recommended for inclusion in new drug applications to licensing authorities [3]. There is therefore interest in the presence and function of transporters in the lung [4,5].

To date transporters in the lung have mainly been studied using gene expression (e.g. polymerase chain reaction [PCR]), immunometric and transporter functional assay methods. For the SLC22A gene family of transporters Horvath et al. [6] have shown OCTN1 and OCTN2 to be present in airway tissues and epithelial cells, from lung donors without preexisting lung disease, using quantitative RT-PCR, immunofluorescence and substrate inhibition assays. The same group has also shown OCT3 to be present in bronchial and vascular smooth muscle cells using similar methods [7]. Nakamura et al. [8] have also shown OCTN1 and OCTN2 to be present in human bronchial epithelial BEAS–2 B cells using RT-PCR. Data obtained in-house (unpublished work) at GlaxoSmithKline (GSK) indicates significant genomic expression of transporters from this gene family in human lung tissue, with a rank order of OCT3 > OCTN1 > OCTN2, and no significant expression of OCT1 or OCT2. In contrast Bleasby et al. [9] have indicated a rank order of mRNA expression of OCTN1 > OCTN2 > OCT3 > OCT1, with no significant expression of OCT2.

For the SLCO gene family of transporters in-house gene expression data (unpublished work) in human lung has indicated noteworthy expression with a rank order of OATP2A1 > 2B1 > 4C1 >

Abbreviations: BCRP, breast cancer resistant protein; BEAS, bronchial epithelium and adenovirus; MDCK, madin darby canine kidney; MDR1, multidrug resistance protein 1 (also known as P-gp); MRM, multiple reaction monitoring; MRP, multidrug resistance-associated protein; NHBE, normal human bronchial epithelial; OATP, organic anion-transporting polypeptide; OCT(N), organic cation transporter (novel); PEPT, peptide transporter; P-gp, permeability glycoprotein 1 (also known as MDR1); SIL, stable isotope label(ed).

* Corresponding author at: Eshelman School of Pharmacy, University of North Carolin at Chapel Hill, 311 Pharmacy Lane, Chapel Hill, NC, 27599-7571, USA.
E-mail address: pcs@email.unc.edu (P.C. Smith).

https://doi.org/10.1016/j.jpba.2018.02.044
0731-7085/

Table 1
Human lung tissue donor information.

Trait	'Lung 2'	'Lung 3'	'Lung 4'	'Lung 5'	'Lung 6'
Age (y)	23	22	33	43	17
Race	Caucasian	Caucasian	Black	Hispanic	Caucasian
Sex	Male	Male	Male	Female	Male
Body Mass Index	24.2	24	37.9	37.8	27.4
Tobacco	No	Yes	Occasional	No	No
Alcohol	No	Occasional	Occasional	No	No
Medication	No	Yes	No	Yes	Yes
White Blood Cell (Billion Cells/L)	10.7	20	15.4	21.1	5.6

3A1 > 4A1, with OATP1A2 not expressed. Bleasby et al. [9], in contrast, have found the order to be OATP2A1 > 3A1 > 2B1 > 4A1 > 4C1 > 1A2.

PEPT2 is the SLC15A2 gene transporter and has been determined by various groups using genetic analysis to be expressed in the lung (GSK [unpublished work]) [9,10]. Takano et al. [11] have shown functional expression of the transporter in the human distal lung epithelial cell line NCI-H441 (H441). Groneberg et al. [12] used immunohistochemical and *ex vivo* uptake studies to show the presence of PEPT2 in human airway tissue.

P-gp (MDR1) (ABCB1 gene transporter), an ATP-binding cassette (ABC) transporter, is found throughout the body [13], including in the lung [4] where its presence has been determined using quantitative RT-PCR [5,13,14]. Lechapt-Zalcman et al. [14], using an immunohistochemical method, found it to localise on the apical surface of airway epithelial cells.

BCRP (ABCG2), also an ABC efflux transporter, has also been found to be present in the lung using quantitative RT-PCR [5,13]. Like P-gp, it is widely distributed throughout the body [13].

MRP9 (ABCC12) was observed in the Bleasby et al. [9] study at high levels but was absent in the GSK analysis of lung mRNA levels (data not shown). Langmann et al. [13] reported low expression of MRP9 in many tissues including the lung. Berg et al. [5] also found the transporter to be very low or absent in healthy lung.

One comprehensive study employing targeted isotope dilution quantitative proteomics with LC–MS/MS for the quantification of transporters in frozen lung has been reported [15]. Many of the transporters mentioned above were quantified in that study. The same group subsequently used their method to quantify transporters in various immortalized human lung cell lines [16] and in a study also employing OCTN1 and MRP1 activity assays with primary cultured human lung cells [17]. Our study, which uses fresh tissue and a less complex sample digestion preparation method, producing a crude membrane fraction, is complimentary and confirmatory to the above studies and provides an interlaboratory comparison of lung transporter quantification by targeted proteomic isotope dilution LC-tandem mass spectrometry.

Herein we employ an adapted targeted quantitative proteomic isotope dilution method, developed in our laboratory [18–21], to quantify membrane transporters in fresh lung from five donors, in four immortalized human lung cell lines and in three control cell lines. We quantify twelve transporters, namely OCT2 (SLC22A2), OCT3 (SLC22A3), OCTN1 (SLC22A4) and OCTN2 (SLC22A5) from the SLC22A cationic uptake transporter family, OATPs (SLCOs) 1A2, 2A1, 2B1 and 4C1 from the SLCO organic anion transporting polypeptide family, the SLC15A oligopeptide transporter PEPT2 (SLC15A2) and three ABC transporters P-gp (ABCB1), BCRP (ABCG2) and MRP9 (ABCC12). The transporters were selected based on the gene expression and immunohistochemical information presented above and on other observations which included 1) in vitro or preclinical data showing involvement in the transport of inhaled drug molecules, 2) known involvement in clinically relevant drug transport and drug–drug interactions in other parts of the body and 3) potential target transporters for respiratory molecules yet to be developed or investigated. MRP9 was included primarily as a negative control.

2. Materials and methods

2.1. Materials

Red Blood Cell (RBC) Lysis Buffer (cat. no. 11814389001, Roche), Ca^{2+} and Mg^{2+}-free phosphate buffered saline containing dispase II (0.2 U/mL, Roche), collagenase/dispase (2 mg/mL, Roche), DNase I, enzyme activity assay kits for membrane purity testing, ammonium bicarbonate, dithiothreitol, β-casein, sodium deoxycholate, iodoacetamide and acetic, trifluoroacetic and formic acids were purchased from Sigma-Aldrich (St. Louis, MO). BD Falcon mesh cell strainers and acetonitrile (HPLC grade) were purchased from Fisher Scientific (Pittsburg, PA). The Pierce® BCA protein assay kit was obtained from VWR International, LLC (Radnor, PA). Ultrapure water was obtained from an in-house Barnstead system (Barnstead/Thermolyne, Dubuque, IA). Trypsin Gold mass spectrometry grade was purchased from Promega (Madison, WI). Crude method development peptides and heavy labeled (^{13}C and ^{15}N on C-terminus R or K) (SIL) proteotypic tryptic peptide standards (SpikeTides™_TQL; purified and quantified) (x 23) (See in supplementary material Table A.1) were purchased from Theracode JPT Inc. (Acton, MA). Solid phase extraction (SPE) cartridges, Strata™-X 33 u Polymeric Reversed Phase (10 mg/mL, part no. 8B-S100-AAK), were obtained from Phenomenex (Torrance, CA).

Lysis buffer for crude membrane isolation, also referred to as hypotonic lysis buffer, was composed of 10 mM Tris (pH 7.5), 10 mM NaC1 and 1 mM $MgC1_2$. Just prior to use the following reagents were added: 1 mM dithiothreitol (DTT), 1% (v/v) aprotinin solution (0.1 U/mL) and 2 mM AEBSF.

MDCKII parental cells were obtained from in house stored culture at GSK, the original ECACC (European Collection of Authenticated Cell Cultures [Porton Down, UK]) stock vial being obtained from Sigma-Aldrich (cat. #85011435). MDCK-MDR1 cells were deposited as an internally generated stable clone, expressing the MDR1 transporter, and obtained from GSK in house repository, BioCat #1507. MDCK-BCRP cells were deposited as an internally generated stable clone, expressing the BCRP transporter, and obtained from GSK in house repository, BioCat #1481. Calu-3 cells were obtained from American Type Culture Collection (ATCC) (Manassas, VA), cat. #HTB-55. A549 cells were obtained from ATCC, cat. #CCL-185. BEAS–2 B cells were obtained from ATCC, cat. #CRL-9609. NHBE cells were obtained from Lonza (Walkersville, MD), cat. #CC2540, and grown in Lonza's *BEGM*™ *BulletKit*™ medium, cat #CC-3171.

2.2. Lung cell isolation

Fresh whole human lung was obtained from the National Disease Research Interchange (Philadelphia, PA) with trachea intact. Donor information is shown in Table 1. Lungs were delivered on ice, and washed with Ca^{2+} and Mg^{2+}-free phosphate buffered