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Functional characterization of the organic cation transporters (OCTs) in human airway pulmonary epithelial cells

Filippo Ingoglia [a,1], Rossana Visigalli [a,1], Bianca Maria Rotoli [a], Amelia Barilli [a], Benedetta Riccardi [b], Paola Puccini [b], Valeria Dall'Asta [a,*]

[a] Dept. of Biomedical, Biotechnological and Translational Sciences (SBiBiT), University of Parma, Via Volturno 39, 43125 Parma, Italy
[b] Preclinical Pharmacokinetics, Biochemistry & Metabolism Dept., Chiesi Farmaceutici, Largo F. Belloli 11/A, 43122 Parma, Italy

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ABSTRACT

Organic cation transporters (OCT1–3) mediate the transport of organic cations including inhaled drugs across the cell membrane, although their role in lung epithelium hasn't been well understood yet. We address here the expression and functional activity of OCT1–3 in human airway epithelial cells A549, Calu-3 and NCl-H441. Kinetic and inhibition analyses, employing [^{3}H]1-methyl-4-phenylpyridinium (MPP+) as substrate, and the compounds quinidine, prostaglandine E2 (PGE_2) and corticosterone as preferential inhibitors of OCT1, OCT2, and OCT3, respectively, have been performed. A549 cells present a robust MPP+ uptake mediated by one high-affinity component (K_m ~ 50 μM) which is identifiable with OCT3. Corticosterone, indeed, completely inhibits MPP+ transport, while quinidine and PGE_2 are inactive and SLC22A3/OCT3 silencing with siRNA markedly lowers MPP+ uptake. Conversely, Calu-3 exhibits both a high (K_m < 20 μM) and a low affinity (K_m > 0.6 mM) transport components, referable to OCT3 and OCT1, respectively, as demonstrated by the inhibition analysis performed at proper substrate concentrations and confirmed by the use of specific siRNA. These transporters are active also when cells are grown under air–liquid interface (ALI) conditions. Only a very modest saturable MPP+ uptake is measurable in NCl-H441 cells and the inhibitory effect of quinidine points to OCT1 as the subtype functionally involved in this model. Finally, the characterization of MPP+ transport in human bronchial BEAS-2B cells suggests that OCT1 and OCT3 are operative. These findings could help to identify *in vitro* models to be employed for studies concerning the specific involvement of each transporter in drug transportation.

1. Introduction

Membrane transporters are known to have a significant impact on the absorption and elimination of a large number of drugs, determining their pharmacokinetic profiles, safety and efficacy [1]. A large family of transporters are the Solute Link Carrier, SLC22A, which are often found in epithelial membranes where they mediate uptake and secretion of organic cations [2]. SLC22A gene family includes electrogenic transporters (*i.e.* OCT1/SLC22A1, OCT2/SLC22A2, and OCT3/SLC22A3) and pH-dependent novel transporters, namely OCTN1/SLC22A4 and OCTN2/SLC22A5 [3]. Although OCTNs may mediate the transport of cationic chemicals, they are most notably known for their ability to mediate the influx of carnitine, and several mutations in the SLC22A5 gene have been identified in patients with primary carnitine deficiency [4]. OCTs are involved in the bidirectional translocation of small (<500 Da) organic cations across the cell membrane. They are endowed with broad, overlapping affinities for a wide range of substrates, including endogenous molecules, such as choline, creatinine and neurotransmitters [5], as well as a variety of xenobiotics [6]. The model substrates for the functional study of OCTs are tetraethylammonium (TEA) and the most specific neurotoxin methyl 4-phenylpiridinium (MPP+) [5,7]. None of the substrates thus far employed interacts only with a single OCT transporter, as demonstrated employing transfected cell models, such as CHO [8], HEK293 [9,10] or xenopus oocytes [11–13]. In non transfected cell models the characterization of OCT transport activity appears further complicated by the lack of specific inhibitors too [5]. The study of OCT transporters has been mainly focused on liver, kidneys, intestine and blood–brain barrier, with the lung remaining largely unchartered terrain, despite its pharmacological relevance [14,15]. Actually, the lung offers a great potential as a portal into the systemic circulation for drugs endowed with difficult oral pharmacokinetics or stability issue. Moreover, since several common inhaled drugs, positively charged at physiological pH, have been reported to interact with OCT [16], the role of these transporters in lung epithelium

Abbreviations: ALI, air–liquid interface culture; EBSS, Earle's Balanced Salt Solution; MPP+, 1-methyl-4-phenylpyridinium; OCT, organic cation transporter; PBS, phosphate-buffered saline solution; PGE_2, prostaglandine E_2; TEER, transepithelial electrical resistance

* Corresponding author at: Unità di Patologia Generale, Dipartimento di Scienze Biomediche, Biotecnologiche e Traslazionali (SBiBiT), Università degli Studi di Parma, Via Volturno, 39, 43125 Parma, Italy. Tel.: +39 0521 033787; fax: +39 0521 033742.
E-mail address: valeria.dallasta@unipr.it (V. Dall'Asta).

[1] F.I. and R.V. equally contributed to this work.

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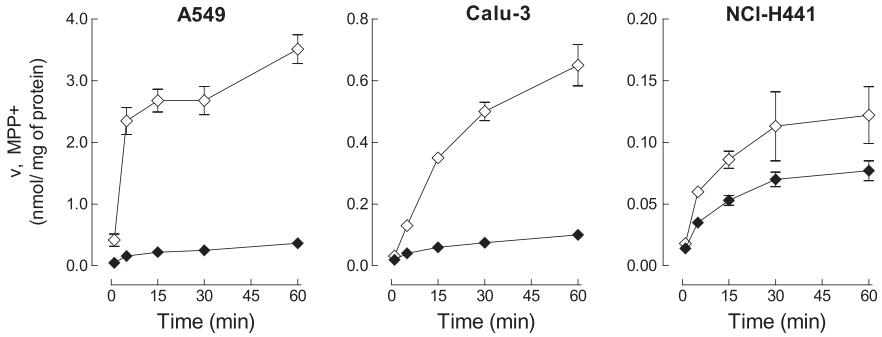

Fig. 1. Time-dependent accumulation of MPP+ in A549, Calu-3 and NCI-H441 cells. Cells were incubated for the indicated times in the transport buffer (see section Material and methods) containing [^{3}H]MPP+ (10 μM; 2 μCi/ml) (open symbols). Non-specific binding of the substrate was estimated by measuring [^{3}H]MPP+ uptake in the presence of an excess of unlabelled substrate (2 mM) (filled symbols). Each point represents the mean ± S.D. of four independent determinations. The experiments have been repeated three times with comparable results.

(especially bronchiolar and alveolar systems) deserves particular attention. Evidence is now emerging that OCTs are involved in transport processes in various cell types of the lung [17,18], and differential expression of the transporters has been highlighted in cell models from different regions [19]. The aim of our study is to identify optimal cell models for studies concerning cationic drug absorption through OCTs in the airways. Thus far, the characterization of OCT-mediated transport in respiratory cell models has been performed employing the fluorescent cation Asp+ as substrate [16,20]. However, since also the choice of the substrate is known to influence the profile of inhibition of OCT-mediated uptake both quantitatively and qualitatively [21], it is conceivable that the use of substrates other than Asp+ may broaden the knowledge about OCT function. In this study we thoroughly characterize OCT transport activity in *in vitro* models of respiratory epithelium (alveolar A549, bronchial Calu-3, and distal lung NCI-H441 carcinoma cells, as well as in bronchial BEAS-2B cells) by means of an integrated approach combining data of mRNA expression with the kinetic and inhibition analyses of MPP+ transport.

2. Material and methods

2.1. Cell cultures

Calu-3, A549, NCI-H441 and BEAS-2B cell lines were obtained from American Type Culture Collection (ATCC, Rockville, MD, USA). Alveolar carcinoma A549 and immortalized BEAS-2B bronchial epithelial cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, high glucose) supplemented with sodium pyruvate (1 mM). NCI-H441, originally derived from lung papillary adenocarcinoma, were cultured in RPMI-1640 (ATCC) and used at passages 36–41. Calu-3 cells, deriving from a human lung adenocarcinoma, were cultured in Eagle's Minimum Essential Medium (EMEM) supplemented with sodium pyruvate (1 mM) and used at passages 45–53. For all cell models, growth medium was supplemented with 10% fetal bovine serum (FBS) and 1% Penicillin/Streptomycin. Cell cultures were routinely cultured under physiological conditions (37.5 °C, 5% CO_2, 95% humidity) in 10-cm diameter dishes.

For growth under air–liquid interfaced culture (ALI) conditions, Calu-3 cells were initially seeded on Cell Culture Inserts (12 mm in diameter, pore size 0.4 μm; Falcon) at the density of 75×10^3 cells/well, with apical and basolateral fluid volumes corresponding to 250 and 700 μl, respectively. After 24 h, the apical fluid was completely removed, and the medium in the basolateral compartment was renewed every other day. Cell cultures were employed after 21 days, when the cell monolayers exhibited "tight" barrier properties, as represented by high transepithelial electrical resistance (TEER > 500 Ohm/cm^2, measured with an epithelial voltmeter (EVOM, World Precision Instruments, FL, USA)). The integrity of cell monolayers was preserved after the experiments.

2.2. qRT-polymerase chain reaction

For the analysis of mRNA expression 1 μg of total RNA, extracted with GenElute Mammalian Total RNA Miniprep Kit (Sigma Aldrich), was reverse-transcribed, and 40 ng of cDNA was amplified as described previously [22]. The following forward and reverse primers were employed: 5′ TGT CAC CGA AAA GCT GAG CC 3′ and 5′ TCC GTG AAC CAC AGG TAC ATC 3′ for SLC22A1/OCT1; 5′ CAT CGT CAC CGC GTT TAA CCT G 3′ and 5′ AGC CGA TAC TCA TAG AGC CAA T 3′ for SLC22A2/OCT2; 5′ AGG TAT GGC AGG ATC GTC ATT 3′ and 5′ GCA GGA AGC GGA AGA TCA CA 3′ for SLC22A3/OCT3; 5′ GCA GCC ATC AGG TAA GCC AAG 3′ and 5′ AGC GGA CCC TCA GAA GAA AGC 3′ for the housekeeping RPL15 (Ribosomal Protein Like 15). The expression

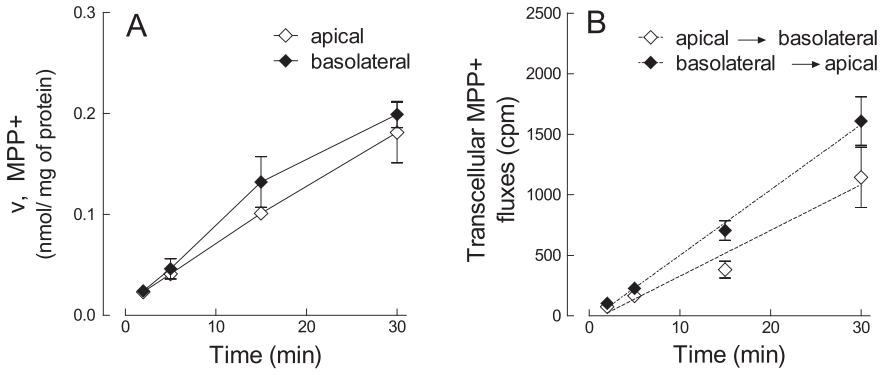

Fig. 2. MPP+ fluxes in Calu-3 ALI. Monolayers of polarized Calu-3 cells grown in air–liquid interface conditions (ALI) were incubated for the indicated time in the transport buffer containing [^{3}H]MPP+ (10 μM; 2 μCi/ml), either added at the apical or at the basolateral side, as indicated. The intracellular MPP+ accumulation (panel A) and the transcellular fluxes of MPP+ (panel B) were determined as described in the section Material and methods. Each point represents the mean ± S.D. of three independent determinations. The experiments have been repeated twice with comparable results.