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Enhanced expression of Organic Cation Transporters in bronchial epithelial cell layers following insults associated with asthma – Impact on salbutamol transport

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ABSTRACT

Increasing evidence suggests Organic Cation Transporters (OCT) might facilitate the absorption of inhaled bronchodilators, including salbutamol, across the lung epithelium. This is essentially scarred and inflamed in asthma. Accordingly, the impact of epithelial insults relevant to asthma on OCT expression and salbutamol transport was evaluated in air-liquid interfaced layers of the human broncho-epithelial cell line Calu-3. These were physically injured and allowed to recover for 48 h or exposed to the pro-inflammatory stimulant lipopolysaccharide (LPS) for 48 h and the aeroallergen house dust mite (HDM) for 8 h twice over 48 h. Increases in transporter expression were measured following each treatment, with the protein levels of the OCTN2 subtype consistently raised by at least 50%. Interestingly, OCT upregulation upon LPS and HDM challenges were dependent on an inflammatory event occurring in the cell layers. Salbutamol permeability was higher in LPS exposed layers than in their untreated counterparts and in both cases, was sensitive to the OCT inhibitor tetraethylammonium. This study is the first to show epithelial injury, inflammation and allergen abuse upregulate OCT in bronchial epithelial cells, which might have an impact on the absorption of their substrates in diseased lungs.

1. Introduction

Inhaled bronchodilators of the β_2-adrenoceptor agonist and M3 muscarinic receptor antagonist classes are routinely used in the management of asthma and chronic obstructive pulmonary disease. In order to exert their pharmacological activity on the airway smooth muscles, the drugs need to overcome the physical barrier provided by the lung epithelium. However, they bear a net positive charge at physiological pH; hence, are expected to exhibit an intrinsic low permeability across biological membranes, which thus places a question mark over the mechanism by which they cross the epithelium.

In vitro data suggests that carrier proteins, particularly those within the Organic Cation Transporter family (OCT), might facilitate their pulmonary absorption (Bosquillon, 2010; Nickel et al., 2016; Salomon and Ehrhardt, 2012). OCT are members of the super family of solute-link carriers SLC22A and comprise the electrogenic OCT1 (SLC22A1), OCT2 (SLC22A2), OCT3 (SLC22A3) sub-types as well as the pH-dependent OCTN1 (SLC22A4) and OCTN2 (SLC22A5) transporters (Koepsell et al., 2007). It is now well established that inhaled bronchodilators have the capacity to inhibit OCT proteins (Bosquillon, 2010; Mukherjee et al., 2012; Salomon et al., 2015) and can be transported by them. The M3 antagonists ipratropium and tiotropium are indeed substrates for OCT1, OCT2 (Hendrickx et al., 2013; Nakanishi et al., 2011), as well as OCTN2 (Nakamura et al., 2010); OCT3 transports only the former (Hendrickx et al., 2013) and OCTN1 has a low affinity for both compounds (Nakamura et al., 2010). Data are less comprehensive regarding the β_2 agonists but nevertheless indicate salbutamol is transported by OCT1 (Salomon et al., 2015) and possibly OCTN2 (Gnadt et al., 2012). In addition, formoterol is likely a substrate for at least one member of the transporter family since its uptake by airway smooth muscles was reduced in presence of OCT inhibitors (Horvath et al., 2007).

Abbreviations: ALI, air-liquid interface; AMC, 7-amino-4-methylcoumarin; BSA, bovine serum albumin; CCL17, chemokine (C-C motif) ligand 17; CCR3, C-C chemokine receptor 3; COX-2, cyclo-oxygenase 2; EDTA, ethylenediaminetetraacetic acid; HBSS, Hank's Balanced Salt Solution; HDM, house dust mite; HPLC-MS/MS, high performance liquid chromatography-tandem mass spectrometry; ICW, In-Cell Western™; LPS, lipopolysaccharide; LY, lucifer yellow VS dilithium salt; OCT, Organic Cation Transporters; P_{app}, coefficient of apparent permeability; PAR-2, protease activated receptor-2; qPCR, quantitative polymerase chain reaction; TARC, thymus and activation regulated chemokine; TEA, tetraethylammonium; TEER, trans-epithelial electrical resistance

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Table 1
Details of primers used for qPCR.

Protein (Gene)	Forward and Reverse sequences	T_m	Annealing temp. (°C)	Efficiency (E value)	Size (bp)
GAPDH	F: 5′-ACAGTCAGCCGCATCTTC-3′ R: 3′-GCCCAATACGACCAAATCC-5′	53.8/53.1	55	1.95	101
18s	F: 5′-CATCTGCCGTTGGAACCTGAAG-3′ R: 3′-CTTGCGGTGTTCTTCCTGGCAT-5′	55.9/57.8	55	1.95	130
OCT1 (SLC22A1)	F: 5′- TGAAGGACGCCGAGAACC-3′ R: 3′-AGGAAGAATACAGAGAAGTGAAGG-5′	55.7/55.6	50	1.84	188
OCT3 (SLC22A3)	F: 5′-CCACTCCACCATCGTCAG-3′ R: 3′-ACACCAAGGCAGGATAGC-5′	53.5/53.0	60	1.98	168
OCTN1 (SLC22A4)	F: 5′-TGTCATCACCCGTAGTTG-3′ R: 3′-ACATACCATTGAAGCCATTG-5′	50.3/50.9	50	2.1	156
OCTN2 (SLC22A5)	F: 5′-GCTACATGGTGCTGCCACTGTT-3′ R: 3′-CTGCCTCTTCAAATCGTCCCTG-5′	57.7/55.4	50	2.4	156
TARC (CCL17)	F: 5′-ACTTCTCCCGGGACTACCT-3′ R: 3′-TCCCTCACTGTGGCTCTTCT-5′	52.9/54.1	58	1.95	111
COX-2	F: 5′-CGGTGAAACTCTGGCTAGACAG-3′ R: 3′-GCAAACCGTAGATGCTCAGGGA-5′	54.9/56.7	60	2.3	156
CCR3	F: 5′-TACTCCCTGGTGTTCACTGTGG-3′ R: 3′-ACGAGGAAGAGCAGGTCCGAA-5′	52.9/54.1	55	1.86	134
iNOS	F: 5′-GCTCTACACCTCCAATGTGACC-3′ R: 3′-CTGCCGAGATTTGAGCCTCATG-5′	54.8/55.8	60	1.94	136
PAR-2 (F2RL1)	F: 5′-CTCCTCTCTGTCATCTGGTTCC-3′ R: 3′-TGCACACTGAGGCAGGTCATGA-5′	53.7/58.1	58	1.90	152

T_m: melting temperature.

Evidence for an OCT-mediated absorption across the airway epithelium is currently limited to salbutamol. The drug was transported to a higher extent in the absorptive than the secretory direction in differentiated epithelial layers of the human bronchial cell lines 16HBE14o- and Calu-3 (Ehrhardt et al., 2005) or the bronchiolar cell line NCI-H441 (Salomon et al., 2015). Moreover, OCT inhibitors reduced the drug absorption in the three cell-culture models when added to the transport medium (Ehrhardt et al., 2005; Haghi et al., 2012; Mamlouk et al., 2013; Salomon et al., 2015). Conversely, a study in normal human bronchial epithelial cell layers concluded that passive diffusion was the primary mechanism for salbutamol transport (Unwalla et al., 2012). Permeability values in the cell layers were nevertheless two orders of magnitude greater, both for salbutamol and the paracellular marker mannitol, than those reported in the cell lines (Ehrhardt et al., 2005; Mamlouk et al., 2013; Salomon et al., 2015). The impact of transporters might therefore have been concealed in such permeable layers.

Transepithelial permeability data for salbutamol have so far been collected in cell layers representing a healthy epithelium. However, in a clinical setting, the drug is most commonly administered to asthmatic lungs. Those are characterised by chronic inflammation, often consecutively to a disproportionate reaction of the pulmonary tissue to inhaled environmental agents. In addition, they exhibit areas of scarring due to defective repair mechanisms following repetitive injury of a hypersensitive epithelium (Holgate, 2011). Epithelial inflammation and wounding might alter OCT expression profile in the lung epithelium, as reported in other tissues. For instance, OCT2 was shown to be downregulated in rat kidneys following injury (Ji et al., 2002) while the renal expression of both OCT1 and OCT2 was decreased upon tissue inflammation induced by administration of the pro-inflammatory agent lipopolysaccharide (LPS) (Heemskerk et al., 2008). In contrast, OCTN1 and OCTN2 levels were raised in rat liver during tissue regeneration post partial resection (Dransfeld et al., 2005). A similar increase in the expression of the latter transporter was also observed in inflamed sections of the intestine in humans (Fujiya et al., 2011). More significantly, as of direct relevance to asthma, variations in OCT1-3 pulmonary expression were measured in rat and murine lungs following their exposure to the model allergen ovalbumin (Lips et al., 2007).

Since inhaled bronchodilators essentially face an inflamed and wounded epithelium when administered to patients, the aim of this study was to evaluate the effect of epithelial insults relevant to asthma on OCT expression in Calu-3 bronchoepithelial cell layers and consequences on salbutamol transepithelial transport. Calu-3 layers maintained at an air-liquid interface (ALI) provide an in vitro model anatomically close to the native bronchial epithelium (Grainger et al., 2006). Importantly, they have been shown to express the same OCT subtypes as normal human bronchial epithelial cell layers (OCT1, OCT3, OCTN1 and OCTN2) on their apical side (Mukherjee et al., 2012) and were deemed a reliable cell culture system for asthma research (Stewart et al., 2012).

ALI Calu-3 layers were physically injured or exposed to environmental agents relevant to asthma, i.e., LPS, a component of Gram negative bacteria wall present in organic dusts and well known to cause lung inflammation (Thorn, 2001) and the aeroallergen house dust mite (HDM), a major trigger for allergic asthma (Gandhi et al., 2013). Changes in OCT expression resulting from the insults were quantified at the gene and protein level by quantitative polymerase chain reaction (qPCR) or In-Cell Western™ (ICW), respectively. Salbutamol absorptive transport was then measured in LPS challenged and control layers in presence of an OCT inhibitor in order to investigate whether the drug is differentially handled by a healthy or an inflamed epithelium.

2. Materials and Methods

2.1. Materials

Calu-3 cells were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). LPS from *E. coli* 0:111B4 was purchased from Sigma-Aldrich (Dorset, UK) and HDM extracts from Indoor Biotechnologies (Warminster, UK). Primers for qPCR analysis were designed using a Beacon Designer Version 7.0 and obtained from Invitrogen, UK (Table 1). The rabbit primary antibodies used for OCT detection by ICW were from Alpha Diagnostics and the anti-GAPDH primary antibody raised in mouse from Sigma-Aldrich. The corresponding secondary antibodies goat anti-rabbit IgG (IRDye® 800CW Conjugate) and goat anti-mouse IgG (IRDye® 680CW Conjugate) were from LI-COR Biosciences UK Ltd. (Cambridge, UK). Salbutamol sulfate was purchased from Alfa Aesar (Heysham, UK). Cell culture media and reagents as well as all other chemicals were from Sigma-Aldrich.