European Journal of Pharmaceutical Sciences 83 (2016) 45–51

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

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

Antibiotic transport across bronchial epithelial cells: Effects of molecular weight, LogP and apparent permeability

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ARTICLE INFO

Article history:
Received 15 April 2015
Received in revised form 24 September 2015
Accepted 7 December 2015
Available online 9 December 2015

Keywords:
Antibiotics
Lung delivery
Calu-3
LogP
Transport
Molecular weight

ABSTRACT

Purpose: The first step in developing a new inhalable formulation for the treatment of respiratory diseases is to understand the mechanisms involved in the absorption of drugs after lung deposition. This information could be important for the treatment of bacterial infection in the lung, where low permeability would probably be beneficial, or a systemic infection, where high permeability would be desirable.
The goal of this study was to evaluate the transport of several antibiotics (ciprofloxacin, azithromycin, moxifloxacin, rifampicin, doxycycline and tobramycin) across human bronchial airway epithelium and to study the influence of molecular weight and LogP on the apparent permeability.
Methods: The experiments were conducted using Calu-3 cells seeded in the apical compartment of 24-well Transwell® inserts. The antibiotics transport was measured in both apical to basolateral (A–B) and basolateral to apical (B–A) directions and the apparent permeability of each antibiotic was calculated.
Results: The A–B transport of ciprofloxacin and rifampicin was independent of the initial concentration in the donor compartment, suggesting the involvement of active transporters in their absorption. Moxifloxacin, doxycycline, azithromycin and tobramycin presented a low absorptive permeation in the A–B direction, indicating that these substances could be substrate for efflux pumps. Generally, all antibiotics studied showed low permeabilities in the B–A direction.
Conclusions: These findings suggest that the inhalation route would be favorable for delivering these specific antibiotics for the treatment of respiratory infection, compared with present oral or intravenous administration.

1. Introduction

Within the past few decades, the lung has received increasing attention as an effective pathway of targeted drug delivery for the treatment of chronic respiratory diseases and as an alternative route for systemic drug administration. Respiratory diseases such as tuberculosis, bronchiectasis, pneumonia, cystic fibrosis and chronic obstructive pulmonary disease benefit from direct administration of drugs to the lung in the form of micronized droplets or solid microparticles. Indeed, for some of these pathologies, inhaled antibiotic therapy has been developed to deliver the drugs directly to the site of action, reducing the dosing requirements, systemic toxicity and side effects (Geller, 2009; Haghi et al., 2014; Traini and Young, 2009). During the development of inhaled formulations it is crucial to understand the fate of the drug candidates after lung deposition, by considering the solubility and lipophilicity of the drugs that could affect bioavailability. The Biopharmaceutics Classification System (BCS) established by Amidon et al. (1995), allows prediction of the in vivo pharmacokinetics of the drugs but was developed for gastrointestinal absorption. Pulmonary administration requires an ad hoc study, taking into account the specific biology of the lung (metabolism, clearance, mucus and surfactant), as well as the characteristics of the formulation (Amidon et al., 1995; Cho et al., 2012; Eixarch et al., 2010).

Several methods have been employed to investigate pulmonary drug absorption such as in vitro cell culture methods, isolated lung perfusion models and in vivo pharmacokinetic analyses (Mobley and Hochhaus, 2001). Among these, in vitro cell cultures may be used as primary screening tools to study the mechanisms involved in the absorption, metabolism and retention time of the drug in the airway epithelium (Borchardt, 1995). A range of in vitro cell culture models of the respiratory epithelia have been employed for the screening of drug candidates and moreover to study the pathological processes involved in lung diseases (Haghi et al., 2014).

Several studies have shown that Calu-3, an adenocarcinoma cell line derived from human bronchial epithelium, forms tight monolayers

Abbreviations: CIP, ciprofloxacin; CIP HCL, ciprofloxacin HCl; AZM, azithromycin; AZM dehydrate, azithromycin dehydrate; MXF, moxifloxacin; MXF HCl, moxifloxacin HCL; RFP, rifampicin; DOX, doxycycline; DOX HCL, doxycycline HCL; TOB, tobramycin.

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http://dx.doi.org/10.1016/j.ejps.2015.12.010
0928-0987/

in vitro, produces secretory components and expresses several efflux and influx transporters (Brillault et al., 2009; Florea et al., 2003; Mathia et al., 2002), suggesting the usefulness of this cell line as an in vitro model to study pulmonary absorption. To the authors' knowledge there are no studies available correlating in vitro results from Calu-3 cell line with human epithelial lining fluid penetration data for this range of drugs.

The objective of this study was to investigate the correlation between physicochemical properties of different antibiotics, such as molecular weight, solubility, LogP and calculated permeability and their transport across Calu-3 cell line. The valuable information obtained from these studies, such as the rate of antibiotics transported across the epithelial cells and the impact of antibiotics physicochemical properties on their transport across the respiratory epithelia would be useful in the future formulation of inhaled antibiotics.

2. Materials and methods

2.1. Materials

Calu-3 cells were purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA). Nonessential amino acids solution, CelLytic M Cell Lysis, protease inhibitor cocktail and dimethyl sulfoxide (DMSO), were purchased from Sigma-Aldrich (Sydney, Australia). Other cell culture reagents including trypsin-EDTA solution (2.5 g/l trypsin, 0.5 g/l EDTA), Dulbecco's modified Eagle's medium (DMEM, without phenol red and L-glutamine, including sodium bicarbonate and 15 mM HEPES), Trypan blue solution (0.4% *w*/*v*), phosphate buffered saline (PBS), L-glutamine solution (200 mM), fetal bovine serum (FBS), and Hanks balanced salt solution (HBSS) were obtained from Invitrogen (Gibco, Invitrogen, Sydney, Australia). Transwell cell culture inserts (0.33 cm^2 polyester, 0.4 μm pore size) were purchased from Corning Costar (Lowell, MA, USA), and all other sterile culture plastic wares were from Sarstedt (Adelaide, Australia). All solvents used were of analytical grade and were supplied by Biolab (Victoria, Australia). Ciprofloxacin (CIP) HCl, azithromycin (AZM) dehydrate and moxifloxacin (MXF) HCl were purchased from Sigma-Aldrich (Sydney, Australia), rifampicin (RFP) was purchased from Hangzhou ICH Imp & Exp Company Ltd. (Hangzhou, China), doxycycline (DOX) HCl was purchased from MP Biomedicals Inc. (Ohio, USA) and tobramycin (TOB) was kindly gifted from Lisapharma S.P.A (Erba, Italy). Rifampicin and the quinolones were protected from a possible degradation from sunlight/other light using aluminum foil, although the experiment duration might have been too short for degradation to play a role.

2.2. Cell culture

The Calu-3 cell line (HTB-55), between passages 35 and 42, was maintained in tissue culture flasks of 75 cm^2 and cultured in pre-warmed DMEM supplemented with 10% (*v*/*v*) fetal bovine serum, 1% (*v*/*v*) nonessential amino acid solution, and 1% (*v*/*v*) L-glutamine solution. Cells were incubated at 37 °C in 5% CO_2 and 95% humidity until confluence was reached. Medium was exchanged every 2 to 3 days, and the cells were passaged weekly, according to ATCC-recommended guidelines. Calu-3 cells were grown at the air interface to allow monolayer differentiation. These conditions have previously been established by Haghi et al. (2010) and Grainger et al. (2006). Cells were seeded at a density of 5.0×10^5 cells/cm^2 in the apical compartment of the 24-well Transwell® cell culture inserts in 100 μl medium and 500 μl medium was added to the basolateral chamber. Subsequently, the medium in the apical chamber was removed after 24 h and was replaced in the basolateral chambers only every 2 to 3 days. The transport experiments were performed between days 11 and 14 after the seeding.

2.3. Viability of Calu-3 cells following exposure to antibiotics

The viability of Calu-3 cells following exposure to different concentrations of antibiotics was assessed using a colorimetric MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2Htetrazolium) in vitro assay, according to the method described by Scalia et al. (2013). Briefly, 5×10^4 cells were seeded per well in a volume of 100 μl into a 96 well plate. Cells were incubated overnight at 37 °C in 5% CO_2 atmosphere.

Concentrations of the antibiotics assessed were as following: CIP up to 50 μM, MXF from 8.0×10^{-4} to 100 μM, RFP from 1.0×10^{-4} to 25 μM, and AZM, DOX, CIP and TOB from 1.0 to 250 μM. After 24 h, 100 μl of increasing concentrations of antibiotics was added to each well. The stock solution of each antibiotic was prepared by dissolving the drug in ethanol or DMSO (<0.5%) and further diluting it with the complete medium to the final ethanol concentration of ≤1%. Untreated and solvent controls were included for each experiment. Plates were incubated for 72 h at 37 °C in a humidified atmosphere with 5% CO_2. Cells were then analyzed for viability. Briefly, 20 μl of CellTiter 96® Aqueous assay (MTS reagent) (Promega, USA) was added to each well and the plates incubated for 3 h at 37 °C in humidified 5% CO_2 atmosphere. Absorbance was measured at 492 nm using a plate reader (Spectramax M2 and Soft Max pro 4.8, Molecular Devices, Sunnyvale, CA, USA). The absorbance value was directly proportional to cell viability (%). Data were expressed as % cell viability [(average absorbance of treated wells / average absorbance of control wells) × 100]. The half maximal inhibitory concentration (IC_{50}) values were defined as the drug concentration that produces a decrease of 50% in cell viability compared to the untreated control. IC_{50} values were calculated by plotting (%) cell viability against the concentrations ($ng \cdot ml^{-1}$) on a logarithmic scale. Data was fitted to the Hill equation using the General Fit function of KaleidaGraph 4.1 software (Synergy Software, Reading, PA, USA), unweighted least-squares regression. Each experiment was performed in triplicate.

2.4. Calu-3 transport experiments of the antibiotics

The transport of antibiotic across cells was measured in both apical to basolateral (A–B) and basolateral to apical (B–A) directions. The selected model antibiotics were dissolved in transport buffer (HBSS, pH = 7.1). The concentration of drug solutions was chosen based on the toxicity of each antibiotic, the solubility of the antibiotic in HBSS and the limit of detection by high performance liquid chromatography (HPLC). The volume of antibiotic solution in the apical compartment was 150 μl for A–B transport, while the receiver compartment was loaded with 350 μl of HBSS. For the B–A transport, 350 μl of antibiotic solution was added to the basolateral compartment and the receiver compartment contained 150 μl of HBSS.

Aliquot of samples was drawn from the receiver compartments at pre-determined time points (30, 60, 120, 180 min) and the sample volume was replaced with fresh pre-warmed HBSS. The cell layer was then harvested for analysis of the drug content inside the cells according to the method previously described (Haghi et al., 2010). Briefly, the cells were harvested and lysed using CelLytic M Cell Lytis buffer, following a centrifugation at 10,000 *g* for 10 min; the supernatant was collected for the analysis of the cellular drug content.

2.5. Sample quantification

Sample concentrations were determined by HPLC. A Shimadzu Prominence UFLC system equipped with an SPD-20A UV–Vis detector, LC-20AT solvent delivery unit, SIL-20A HT Autosampler (Shimadzu Corporation, Japan) was used for the analysis. The column, wavelength and mobile phase composition for each antibiotic studied are shown in Table 1. Flow rate was 1.0 ml/min and the injection volume was 70 μl