Journal of Pharmaceutical Sciences xxx (2017) 1-7

Contents lists available at ScienceDirect

Journal of Pharmaceutical Sciences

journal homepage: www.jpharmsci.org

Pharmacokinetics, Pharmacodynamics and Drug Transport and Metabolism

Active Mediated Transport of Chloramphenicol and Thiamphenicol in a Calu-3 Lung Epithelial Cell Model

Siti N. Nurbaeti [1, 2], Jean-Christophe Olivier [1, 2], Christophe Adier [1, 3], Sandrine Marchand [1, 2, 3], William Couet [1, 2, 3], Julien Brillault [1, 2, *]

[1] INSERM U-1070, Pôle Biologie Santé, Poitiers, France
[2] Université de Poitiers, UFR Médecine-Pharmacie, Poitiers, France
[3] Laboratoire de Toxicologie-Pharmacocinétique, CHU de Poitiers, Poitiers, France

A R T I C L E I N F O

Article history:
Received 3 August 2017
Revised 15 November 2017
Accepted 28 November 2017

Keywords:
pulmonary delivery/absorption
cell culture
ABC transporters
permeability

A B S T R A C T

Pulmonary administration enables high local concentrations along with limited systemic side effects but not all antibiotics could be good candidates. In this perspective, diffusion of the antibiotic chloramphenicol (CHL) and thiamphenicol (THA) through the lung has been evaluated to reassess their potential for pulmonary administration. The apparent permeability (Papp) was evaluated with the Calu-3 cell model. The influence of drug transporters was assessed with the PSC-833, MK-571, and KO-143 inhibitors. The influence of CHL and THA on the cell uptake of rhodamin 123 and fluorescein was also evaluated. Absorptive Papp of CHL and THA was concentration independent with CHL Papp 4 times higher than that of THA. Secretory Papp of CHL was concentration independent, whereas it was concentration dependent for THA with an efflux ratio of 3.6 for the lowest concentration. The use of inhibitors suggested that CHL and THA were substrates of efflux transporters but with a low affinity. In conclusion, the permeability results suggest that the pulmonary route may offer a biopharmaceutical advantage only for THA. Owing to the influence of drug transporters, a higher concentration in the lung than in the plasma is expected mostly for THA, whatever the route of administration.

Introduction

The rapid emergence of resistant bacteria is a complex and major threat, endangering the efficacy of antibiotics (ATBs). The development of new ATBs is urgently needed but has been almost abandoned by the industry due to reduced economic incentives. Therefore, old forgotten ATBs constitute a valuable alternative to eradicate emerging bacteria becoming resistant to most of the currently used ATB.[1] The use of such ATBs had been limited mainly because of lower tolerability, compared to the new generations of ATBs. However, by choosing the best dosing regimen and route of administration, side effects could be limited, and a new therapeutic interest could be given to these old drugs. This is particularly obvious in the case of infectious lung diseases where the pulmonary route of administration could give a real advantage for the ATB treatments where high local concentrations could be achieved. Suboptimal exposure, that favors the development of resistances, could be then avoided along with a limited systemic concentration and decreased side effects. In this perspective, the reuse of chloramphenicol (CHL) has been gaining interest for the treatment of infectious lung diseases. CHL is an old broad-spectrum ATB discovered in 1947 and effective against many Gram-positive and Gram-negative bacteria.[2] Its activity is high against methicillin-resistant *Staphylococcus aureus*, fair against *Klebsiella pneumoniae or Streptococcus pneumonia*, and poor against *Acinetobacter baumannii* or *Pseudomonas aeruginosa*.[3,4] Owing to its effectiveness, availability, and low price, it is still frequently used in the developing world. Regarding the toxicity, dose-related and reversible bone marrow suppression is the most common. Another toxic effect, rare but fatal, is aplastic anemia which occurs in 1 in 25,000-40,000 patients.[5] Owing to these adverse effects, CHL is rather recommended for second-line treatments. Thiamphenicol (THA) is a methane-sulfonate derivative of CHL, with a comparable antimicrobial spectrum and activity. It has been widely used in Europe and Japan, especially for respiratory tract infections.[6] THA has been also associated with reversible bone marrow suppression but never with fatal aplastic anemia. With the lack of new ATBs, phenicol drugs should be re-evaluated, especially in the case of lung infections where the pulmonary route of administration could lead to high local concentrations and limited adverse effects. However, the

* *Correspondence to:* Julien Brillault (Telephone: +33-549454944; Fax: +33-549454378).
E-mail address: Julien.brillault@univ-poitiers.fr (J. Brillault).

https://doi.org/10.1016/j.xphs.2017.11.021
0022-3549/© 2017 American Pharmacists Association®. Published by Elsevier Inc. All rights reserved.

pulmonary route is to be chosen only for drugs with physicochemical properties that favor a longer residence time and a higher concentration in the lung than in the plasma compartment. The main factor that affects the drug absorption from lung to plasma is the epithelial cell permeability. Several *in vivo* studies have shown a higher concentration of ATBs in the lung compartment after the pulmonary administration of drugs with a low membrane permeability such as colistin or aztreonam.[7-9] For higher permeability drugs such as fluoroquinolones, *in vivo* studies have demonstrated that their concentrations equilibrated quickly between the lung and plasma compartments whatever the route of administration, suggesting a limited advantage of the pulmonary route.[10] A second factor that affects drug concentrations in the lung is the presence of drug transporters at the bronchoalveolar epithelium. Indeed, several ATP-binding cassette transporters, such as P-glycoprotein (P-gp), multidrug resistance-associated proteins (MRPs) and breast cancer resistance protein (BCRP), have been reported to be expressed in lung tissue, and ATBs approved by the U.S. Food and Drug Administration for inhalation, such as fluoroquinolones or aztreonam, have been shown to be substrates of such drug transporters.[11] If ATBs are substrates of efflux transporters, different concentrations are expected in the lung compartment and plasma. As evidence on the drug transporter presence in the lung is raising, a better understanding of their contributions in the drug distribution is needed.[12] In this regard, information about permeability and efflux transport for CHL and THA does not exist to date in the literature, with the last studies dating mostly from the early 80s. Knowledge about ATB membrane permeability is necessary in order to choose the best route of administration in the case of lung infection treatment or to design a suitable formulation for the pulmonary administration. The objective of this study was to assess the permeability, drug uptake, and efflux transport of CHL and THA. The *in vitro* Calu-3 cell model was chosen to perform these experiments as it is a well-established model for drug transport, where the expression of the main drug transporters, such as P-gp, MRPs, and BCRP, has been demonstrated.[11,13]

Materials and Methods

Chemicals

THA (98% pure), CHL (99% pure), dimethyl sulfoxide (DMSO), MK-571, KO-143, Triton X-100, sodium fluorescein, and rhodamin 123 (RHO) were purchased from Sigma-Aldrich. PSC-833 was kindly supplied by Novartis (Basel, Switzerland). Hanks' balanced salt solution and phosphate-buffered saline (PBS) pH 7.4, sodium bicarbonate, Dulbecco's modified Eagle's medium-F12, fetal bovine serum, and HEPES buffer were supplied from PAN Biotech GmbH (Aidenbach, Germany). Transwell® clear polyester membranes with a 1.12-cm^2 area and a pore size of 0.4 μm were obtained from Corning Costar (Corning, NY). Nunclon Delta Surface 96-well plates were supplied from Thermo Fisher Scientific (Roskilde, Denmark). All other reagents were of analytical grade.

Calu-3 Cell Culture

The Calu-3 cells were purchased from the American Type Culture Collection (HTB55™; ATCC®, Rockville, MD). The cells between passages 41 and 60 were cultured in Dulbecco's modified Eagle's medium-F12 medium with 2.2 g/L bicarbonate and supplemented with L-glutamine (2 mM) and 10% (vol/vol) fetal bovine serum. The cells were seeded at a density of 15×10^4 cells/well into Transwell® inserts (12-well plates, 12-mm diameter inserts, 0.4-μm pore size, tissue culture treated, polyester membrane) with a volume of 0.5 mL medium in the apical (Ap) compartment and of 1 mL in the basolateral (Bl) compartment. Cells were then cultured under air-interface conditions at 37°C in air with 5% CO_2 and 90%-95% relative humidity, for 15 days before the experiment with the renewal of the basal compartment with 1.5 mL of medium every other day.

Time Effect

Solutions of 10 and 500 μg/mL of CHL or THA were prepared in a transport medium (TM: Hanks' balanced salt solution supplemented with 10 mM HEPES buffer). The flux of drug through the Calu-3 cell monolayer was evaluated in apical-to-basolateral (Ap-Bl) and basolateral-to-apical (Bl-Ap) directions. On the day of the experiment, inserts with cells were first washed 3 times 10 min with the TM in both compartments. Then, the acceptor compartment was filled with the TM (1.5 mL for Bl compartment or 0.5 mL for Ap compartment). Solution of CHL or THA was added in the donor compartment (1.5 mL for Bl compartment or 0.5 mL for Ap compartment), and the cells returned to the incubator. At 60, 120, and 180 min, 150 μL from the acceptor compartment was sampled and replaced with the same volume of the TM. The samples were stored at −80°C until analysis. To check for the integrity of the Calu-3 cell barrier, the transport of fluorescein (FLU) was carried out at the end of the experiments in Ap-Bl direction. The Calu-3 cells were rinsed once with the TM in both compartments, and the cells were incubated with the TM in the Bl compartment and 10 μg/mL of sodium FLU in the TM in the Ap compartment. Samples were collected after 60 min in the incubator, and the concentration of FLU was evaluated with a fluorescent plate reader (Tecan Infinite 200 pro; Tecan Group Ltd., Männedorf, Switzerland), and the apparent permeability (Papp) value for FLU was calculated. A threshold Papp value of 0.7×10^{-6} $cm \cdot s^{-1}$ was retained for the tight junction integrity rejection parameter for all experiments. This corresponds to the transfer of <0.5% of the initial amount in the Ap compartment.[14]

Concentration Effect

The experiments were conducted as described previously with the following modifications: transport experiments were evaluated with 5, 10, 50, 100, 250, and 500 μg/mL concentrations for CHL and THA. Only one sample was collected after 60 min because time experiments demonstrated linearity over 180 min, and samples were stored at −80°C until analysis. The Papp (cm/s) was calculated using Equation 1 where Q (μg) is the amount of drug in the acceptor compartment after a time Δt (s), S is the insert membrane surface (1.12 cm^2), and [Co] is the initial drug concentration in the donor compartment (μg/mL).

$$\mathrm{Papp} = \frac{Q}{[Co].\Delta t.S} \tag{1}$$

Inhibition Studies

Stock solutions of 300 μM PSC-833, MK-571, and KO-143 were prepared in DMSO and stored at −20°C. Cells were first equilibrated and rinsed 2 times with the TM for 10 min each and then rinsed a third time with the TM in the presence of inhibitors in both compartments. Transport experiments were then realized as described previously with 10 μg/mL of CHL, THA, FLU, or RHO in the donor compartment and in the presence of 3 μM inhibitors in both compartments. Control experiments without inhibitors were done in the TM with 1% DMSO. Cells were incubated for 60 min, and samples were collected in the acceptor compartment. The samples were stored at −80°C until analysis.