Development of a Novel Lung Slice Methodology for Profiling of Inhaled Compounds

ERICA BÄCKSTRÖM,[1,2] ANDERS LUNDQVIST,[2] ELIN BOGER,[2,3] PETTER SVANBERG,[2] PÄR EWING,[2] MARGARETA HAMMARLUND-UDENAES,[1] MARKUS FRIDÉN[1,2]

[1]Translational PKPD, Department of Pharmaceutical Biosciences, Uppsala University, Uppsala 751 24, Sweden
[2]Respiratory, Inflammation and Autoimmunity Innovative Medicines, AstraZeneca R&D, Mölndal 431 83, Sweden
[3]School of Engineering, University of Warwick CV4 7AL, Coventry, United Kingdom

Received 8 May 2015; revised 23 June 2015; accepted 23 June 2015

Published online in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.24575

ABSTRACT: The challenge of defining the concentration of unbound drug at the lung target site after inhalation limits the possibility to optimize target exposure by compound design. In this study, a novel rat lung slice methodology has been developed and applied to study drug uptake in lung tissue, and the mechanisms by which this occurs. Freshly prepared lung slices (500 μm) from drug-naive rats were incubated with drugs followed by determination of the unbound drug volume of distribution in lung ($V_{u,lung}$), as the total concentration of drug in slices divided by the buffer (unbound) concentration. $V_{u,lung}$ determined for a set of inhaled drug compounds ranged from 2.21 mL/g for salbutamol to 2970 mL/g for dibasic compound A. Co-incubation with monensin, a modulator of lysosomal pH, resulted in inhibition of tissue uptake of basic propranolol to 13%, indicating extensive lysosomal trapping. Partitioning into cells was particularly high for the cation MPP+ and the dibasic compound A, likely because of the carrier-mediated transport and lysosomal trapping. The results show that different factors are important for tissue uptake and the presented method can be used for profiling of inhaled compounds, leading to a greater understanding of distribution and exposure of drug in the lung. © 2015 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci

Keywords: pulmonary; tissue partition; pharmacokinetics; pulmonary drug delivery; distribution

INTRODUCTION

Drug delivery by inhalation for the treatment of respiratory diseases is advantageous as it allows direct access to the site of action with a minimum of systemic exposure and side effects.[1,2] There is however limited knowledge of the local lung tissue pharmacokinetics (PK) after inhalation and systemic PK data cannot be assumed to predict the clinical performance of inhaled compounds as it is the concentration in the lung that drives the therapeutic response.[3] Methodologies to estimate the unbound target site concentration in the lung has been lacking.

The design of inhaled drugs typically aims at minimizing systemic exposure by having low oral bioavailability and high systemic clearance. Furthermore, controlled pulmonary absorption of the lung deposited dose allows a minimum of systemic exposure of the drug, while allowing a local lung exposure to be maintained to exhibit pharmacological activity throughout the dosing interval. This concept is referred to as lung or airway selectivity. Over time, two main strategies for designing controlled absorption from the lung have emerged, where absorption rate is either limited by slow drug particle dissolution or by the affinity of the dissolved compound to lung tissue.[4] Examples of inhaled compounds that are believed to be retained in the lung as slowly dissolving depots are corticosteroids such as fluticasone propionate and fluticasone furoate.[5] Long-acting inhaled β-adrenergic bronchodilators on the other hand, are believed to be retained in the lung in a dissolved form owing to their high solubility and pronounced affinity to lung tissue.[4]

Affinity to lung tissue, including lysosomal trapping of basic compounds, plays an important role in the design of inhaled drugs to facilitate lung selectivity.[4] Lysosomes, which are particularly abundant in lung tissue,[6] are acidic organelles (pH 4–5) that play a key role in various metabolic processes, such as the turnover of phospholipids, the breakdown of endogenous waste products, autophagy, and apoptosis.[7] Sequestration of basic compounds inside lysosomes, that is, lysosomal trapping, results from basic compound in the cytosol diffusing into the lysosome where it becomes charged. The higher proportion of the non-permeable ionized species then restricts back-diffusion.

In the absence of methodologies to effectively determine the unbound pharmacologically active lung target site concentration, *in vivo* PK studies have instead focused on measuring the amount of drug in whole lungs at different time points after intratracheal drug administration or inhalation.[4] In these studies, the determined drug amount, which is referred to as the total lung concentration, makes no distinction between extracellular and intracellular compartments, bound or unbound drug let alone the local variations that one may expect after inhalation. The rate of absorption from the lung has been correlated with cellular permeability determined in, for example, with Caco-2 cells on Transwell® plates,[8] however membrane

Abbreviations used: $C_{u,cell}$, intracellular unbound drug concentration; $C_{u,lungISF}$, unbound drug concentration in the lung interstitial fluid; $f_{u,hD}$, fraction of unbound drug in diluted lung homogenate; $f_{u,lung}$, fraction of unbound drug lung homogenate; LDH, lactate dehydrogenase; $K_{p,u}$, total lung/unbound plasma concentration at steady state; $K_{p,uu,cell}$, unbound drug partitioning coefficient of the cell; MPP+, 1-methyl-4-phenylpyridinium; PBPK, physiologically based pharmacokinetic; PD, pharmacodynamic; PK, pharmacokinetic; V_0, volume of the airspaces in the lung slices; $V_{u,lung}$, unbound drug volume of distribution in the lung.

Correspondence to: Erica Bäckström (Telephone: +46-317761516; Fax: +46-317763700; E-mail: erica.backstrom@astrazeneca.se)

This article contains supplementary material available from the authors upon request or via the Internet at http://wileylibrary.com.

Journal of Pharmaceutical Sciences
© 2015 Wiley Periodicals, Inc. and the American Pharmacists Association

permeability does not always seem to be the major limiting factor in the process. For instance insulin, which has low permeability, is absorbed in lung tissue within minutes after inhalation.[9] The anti-muscarinic agents ipratropium and tiotropium with extremely low passive permeability are similarly absorbed,[4] whereas moderately sized lipophilic drugs can be effectively retained.

In the present study, we propose a new lung slice methodology to study non-specific binding and lysosomal uptake as facilitators for lung retention, as an important method for profiling of inhaled compounds. To enable the study of lysosomal trapping and/or carrier-mediated uptake in more detail, viable tissue with the retained complexity and integrity of the lung structure is required, and freshly prepared lung slices fulfil these criteria. Experiments with brain slices show that drug distribution into cells include actual binding, lysosomal trapping, and carrier-mediated uptake.[10] Other approaches to measure tissue affinity include equilibrium dialysis or ultrafiltration of lung homogenates.[11,12] Homogenization of tissues has the disadvantage of disrupting the normal drug compartmentalization in the cells, including the pH differences, thereby precluding the possibility to study lysosomal trapping. The use of equilibrium dialysis or ultrafiltration of lung homogenates are not standard practices in pharmacokinetic/pharmacodynamic (PK/PD) evaluation of inhaled compounds because of the potentially large errors in the estimates of free drug concentration.[11]

Technical aspects of the new lung slice methodology such as viability and experimental conditions were investigated in the presented study, followed by characterization of lysosomal trapping of basic drugs, and application of the method to study intracellular accumulation of common inhaled drugs. The presented methodology can facilitate and improve the knowledge of PK/PD relationships of lung targeted drugs as well as improve the process of optimizing drug compounds for inhalation.

MATERIALS AND METHODS

Compound Selection

Three model compounds were chosen to characterize the lung slice method, basic propranolol, a non-selective beta-blocker, acidic indomethacin, a non-steroidal anti-inflammatory drug, and neutral diazepam, a highly permeable compound. An additional 10 compounds with relevance to lung delivery, from different classes with diverse pK_a and lipophilicity, were chosen as a representation of relevant inhaled drug compounds. The compounds were the bases formoterol, salbutamol, and salmeterol, the cations *1*-methyl-4-phenylpyridinium (MPP+), ipratropium, and tiotropium, the neutral compounds budesonide and fluticasone propionate, the zwitterion indacaterol, and one dibase (compound A).

Chemicals

Ipratropium was obtained from Sigma–Aldrich (St. Louis, Missouri). The other drugs were obtained from the AstraZeneca compound library. All other chemicals were of analytical grade and all solvents were of HPLC grade.

Animals

Male Wistar-Han rats (Harlan, Horst, The Netherlands) weighing 300–400 g were used in the present study. Animals were housed in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited animal facility in groups of six individuals at 18°C–22°C under a 12-h light/dark cycle with access to water and chow *ad libitum* for at least 5 days prior to the experiments. The study was approved by the Animal Ethics Committee of Gothenburg (234–2011, 134–2013, and 137–2014).

Preparation of Lung Slices

Drug-naïve rats were anesthetized with isoflurane (FORENE®; Abbott Scandinavia AB, Solna, Sweden) and exanguinated after injection of heparin (0.4 mL; LEO Pharma, Malmö, Sweden) to the heart. The pulmonary circulation was perfused with 37°C saline (15 mL, 0.9% NaCl) and a 37°C solution of low melting point agarose solution (1.5% agarose in saline, 7 mL; Apollo Scientific, Manchester, UK) via the pulmonary artery. Low melting point agarose allows the temperature of the agarose solution to be reduced to body temperature after boiling without gelling of the agarose.[13] The artery was then sealed with a suture to prevent leakage. The lung was inflated via the trachea through instillation of a 37°C solution of the low melting point agarose (1.5% agarose in saline, 4.5 mL) and subsequently sealed with a suture. After removal, the lung and heart were immediately immersed in ice-cold buffer [124.5 mM NaCl, 0.8 mM $MgSO_4$, 25.2 mM HEPES, 5.4 mM KCl, 1.8 mM $CaCl_2$, 10 mM glucose (pH 7.4 at 37°C)] and placed on ice for at least 20 min in order to solidify the agarose to enable slicing. The described buffer is referred to as "buffer." The lung lobes were dissected free and mounted with cyanoacrylate glue onto the tray of a DTH-Zero 1 Microslicer (Dosaka, Kyoto, Japan). Slices (500 μm) were prepared using cutting speed 6 and frequency 7. Slices were kept in ice-cold buffer until start of incubation.

General Incubation and Sample Preparation Protocol

Three lung slices (~150 mg in total) were incubated in 15 mL buffer containing the drug(s) of interest in 80 mm flat-bottomed glass dishes. The glass dishes were covered with plastic paraffin film (Bemis, Neenah, Wisconsin) and glass lids and placed in a humidified box inside the Forma Orbital Shaker 420 (Thermo Fisher Scientific, Waltham, Massachusetts), set at 37°C with a rotation speed of 45 rpm. At predefined time points (depending on experimental set up), all three slices were removed simultaneously, dried briefly on filter paper, and weighed together before homogenization in 9 volumes (w/v) of buffer with an ultrasonic probe (Sonifier 250; Branson Ultrasonics, Danbury, Connecticut). The incubation buffer was sampled (200 μL) from the glass dish to a tube containing blank lung homogenate (200 μL) that had been prepared with 4 volumes (w/v) of buffer. The samples were stored at −20°C until analysis with LC–MS/MS.

Assessment of Viability of the Lung Slices

To assess the viability of the slices, the amount of lactate dehydrogenase (LDH) released from the slices into the incubation buffer relative the total amount in slices was measured using a CytoTox 96® Non-Radioactive Cytotoxicity assay kit, according to the manufacturer's instructions (Promega, Madison, Wisconsin). Briefly, lung slices were incubated and samples of buffer (experimental value) and lung slices (slice value) were taken at different time points up to 48 h. At the time of sampling, all slices were removed, homogenated together, and then the homogenate was mixed with Triton X-100 (Amresco, Solon, Ohio)

 DOI 10.1002/jps.24575

Download English Version:

https://daneshyari.com/en/article/2484287

Download Persian Version:

https://daneshyari.com/article/2484287

Daneshyari.com