Lung pharmacokinetics of inhaled and systemic drugs: A clinical evaluation

Muhammad Waqas Sadiq1,2,3, Olaf Holz4,5, Birthe D. Ellinghusen4, Cornelia Faulenbach4, Meike Müller4, Philipp Badorrek4, Ulf G. Eriksson1,2,3, Markus Fridén6,7,8,9, Stina Stomilovic6,7,8, Anders J. Lundqvist6,7,8,9, Jens M. Hohlfeld4,5,11

Background and Purpose: Human pharmacokinetic studies of lung-targeted drugs are typically limited to measurements of systemic plasma concentrations, which provide no direct information on lung target-site concentrations. We aimed to evaluate lung pharmacokinetics of commonly prescribed drugs by sampling different lung compartments after inhalation and oral administration.

Experimental Approach: Healthy volunteers received single, sequential doses of either inhaled salbutamol, salmeterol and fluticasone propionate (n = 12), or oral salbutamol and propranolol (n = 6). Each participant underwent bronchoscopies and gave breath samples for analysis of particles in exhaled air at two points after drug administration (1 and 6, 2 and 9, 3 and 12, or 4 and 18 h). Lung samples were taken via bronchosorption, bronchial brush, mucosal biopsy and bronchoalveolar lavage during each bronchoscopy. Blood samples were taken during the 24 h after administration. Pharmacokinetic profiles were generated by combining data from multiple individuals, covering all sample timings.

Key Results: Pharmacokinetic profiles were obtained for each drug in lung epithelial lining fluid, lung tissue and plasma. Inhalation of salbutamol resulted in approximately 100-fold higher concentrations in lung than in plasma. Salmeterol and fluticasone concentration ratios in lung versus plasma were higher still. Bronchosorption- and bronchoalveolar-lavage-generated profiles of inhaled drugs in epithelial lining fluid were comparable. For orally administered drugs, epithelial-lining-fluid concentrations were overestimated in bronchoalveolar-lavage-generated profiles.

Conclusion and Implications: Combining pharmacokinetic data derived from several individuals and techniques sampling different lung compartments enabled generation of pharmacokinetic profiles for evaluation of lung targeting after inhaled and oral drug delivery.

Abbreviations: BAL, bronchoalveolar lavage; ECGs, electrocardiograms; ELF, epithelial lining fluid; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; HILIC, hydrophilic interaction LC; LC–MS/MS, LC coupled with tandem mass spectrometer; MRM, MS data (multi-reaction monitoring); PEx, particles in exhaled air; PExA, device for particlesampling (particles in exhaled air); PK/PD, pharmacokinetics/pharmacodynamics.
1 | INTRODUCTION

The inhaled route of administration promises selective delivery of lung-targeted drugs to their target organ, with minimal systemic side effects. Factors including lung deposition and the physicochemical properties of the compound are known to be important for the pharmacological efficacy of inhaled drug products (Borghardt et al., 2018). However, just how effectively a given inhaled or oral drug targets the lung is difficult to establish because of experimental limitations associated with making measurements of active drug at the target sites within the complex structures of the lung. Therefore, in most standard clinical settings, pharmacokinetic data are restricted to systemic plasma concentrations. Lung targeting refers to selective, high (free and pharmaceutically active) drug exposure in different lung compartments in comparison to systemic exposure (Torchilin, 2000). In this study, we are advancing and evaluating methodologies for sampling the lung for evaluation of regional lung targeting.

Several techniques for obtaining lung samples can be utilized for pharmacokinetic analysis, enabling access to different regions and compartments of the lung (Forbes et al., 2011). Mucosal biopsy and bronchial brushing, both performed via bronchoscopy, are established methods that typically sample tissue from the bronchi at generations four to six of the airway, the former sampling the mucosa and the latter sampling epithelial cells and epithelial lining fluid (ELF). Bronchoalveolar lavage (BAL), meanwhile, retrieves ELF from all generations from the 4th to 5th generation down to the alveoli (20–23 generation) thus combining central and peripheral lung with a dominance of the peripheral lung (Haslam & Baughman, 1999). However, the BAL procedure dilutes these ELF samples to an unknown extent, making meaningful estimates of drug concentrations challenging (despite available correction methods) (Forbes et al., 2011). Bronchosorption can be used to measure drug levels directly in ELF samples from generations four to six of the airway, without any dilution, and was reported to have greater sensitivity to BAL in a clinical study of inflammatory biomarkers in ELF (Leaker et al., 2015). Finally, particles in exhaled air (PEX), which form when collapsed small airways reopen during inhalation, comprise undiluted ELF from the small airways and can be sampled by impaction (Bake et al., 2019; Schwarz et al., 2010). Whether this novel, non-invasive method can measure non-volatile drug levels in ELF has not yet been assessed.

The present study had three aims. First, to assess the technical feasibility of measuring drug concentrations in spatially defined regions of the human lung after inhalation or oral administration. Second, to assess whether the regional lung targeting achieved by several commonly prescribed inhaled drugs can be evaluated through sampling ELF from different compartments of the lung. Third, to explore the utility of various lung-sampling methods for pulmonary pharmacokinetic analysis, including bronchosorption, BAL, mucosal biopsies, bronchial brushing and PEx.

2 | METHODS

2.1 | Overview

This was a single-centre, exploratory study conducted in healthy, non-smoking men and women (ClinicalTrials.gov identifier: NCT03524066). The study took place between April 2018 and December 2018 at the Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM), Hannover, Germany. It was conducted in accordance with the principles of the Declaration of Helsinki and the Good Clinical Practice Guideline of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. An independent ethics committee at Hannover Medical School, Hannover, Germany, reviewed and approved the study protocol. All participants freely gave their written informed consent before starting the study.

2.2 | Inclusion criteria

Men or women aged 18–50 years were potentially eligible for inclusion. Women must have been either not pregnant and not nursing, of
non-childbearing potential, or of childbearing potential and using an effective method of contraception during the entire study. Participants were required to have normal lung function, that is, with a predicted forced expiratory volume in 1 s (FEV1) ≥ 80% of normal and an FEV1 to forced vital capacity (FVC) ratio >70%. Participants must also have been non-smokers, with a history of less than one pack per year for the past 5 years, and have had a body mass index of 18–32 kg m⁻².

Exclusion criteria were past or present disease that may have affected the outcome of the study, including pulmonary diseases such as asthma, tuberculosis, bronchiectasis, or cystic fibrosis; regular intake of any prescribed or over-the-counter medication, with the exceptions of paracetamol for pain relief, oral contraceptive medication, hormonal replacement therapy, and dietary and vitamin supplements; history of clinically relevant allergy; intolerance or contraindications to the study drugs or to sedation during bronchoscopy; infections of the lower respiratory tract in the 4 weeks before, or during, the study; any clinically relevant abnormal findings in physical examination, clinical chemistry, haematology, urinalysis, vital signs, lung function or electrocardiograms (ECGs) at screening that may have put the volunteer at risk because of study participation or may have influenced the results of the study or the volunteer’s ability to participate in the study; history of drug or alcohol abuse.

2.3 Study design

Participants were assigned to one of three cohorts (Figure 1). The ‘pilot cohort’ (n = 2) was conducted first to confirm the suitability of the study methods. This cohort and the subsequent ‘inhalation cohort’ (n = 12) received single doses of commercially available salbutamol 200 μg (836 nmol), salmeterol 50 μg (120 nmol), and fluticasone propionate 500 μg (1125 nmol) by inhalation back-to-back. Salbutamol (Sultanol Dosier-Aerosol, GlaxoSmithKline, München, Germany) was inhaled from a metered-dose inhaler via spacer (VORTEX Inhalierhilfe, PARI, Starnberg, Germany). Salmeterol and fluticasone propionate (combined formulation) were inhaled from a dry-powder dual inhaler (Viani forte, GlaxoSmithKline, München, Germany). The ‘oral cohort’ (n = 6) received single, sequential doses of oral salbutamol 8 mg (33.43 μmol) (VOLMAC retard, Dexcel Pharma, Alzenau, Germany) and oral propranolol 40 mg (154.2 μmol) (Dociton, Mibe, Brehna, Germany). In each cohort, all study drugs were administered within approximately 3 min of each other, under strict professional supervision. All participants also received midazolam and lidocaine during the bronchoscopy (see below). The drugs tested in the study were chosen to cover a range of drug classes that are typically used to treat airway diseases and that have a spectrum of physicochemical properties, rather than to generate safety or efficacy data. Randomisation and blinding were not performed because they were not necessary for the investigation of lung-sampling methods for pulmonary pharmacokinetic analysis.

All participants underwent two bronchoscopies for lung sampling at intervals (either 1 and 6 h, 2 and 9 h, 3 and 12 h, or 4 and 18 h) after drug administration. Breath samples were taken immediately before each bronchoscopy. Blood samples were taken during the 24 h after drug administration.

Intensive exercise had to be avoided on the day before bronchoscopy. Participants were not allowed to eat or drink during the 12 h before the procedure. Medications other than paracetamol (as a mild analgesic), those related to the bronchoscopy, and ipratropium...
bromide for rescue bronchodilator therapy after bronchoscopy were prohibited.

2.4 Procedures

2.4.1 Bronchoscopy

Bronchoscopy was performed according to standard operating procedures consistent with international recommendations (Bleecker et al., 1991; British Thoracic Society Bronchoscopy Guidelines Committee, 2001) using bronchoscopes with a 2.0 mm working channel (Olympus Europa SE & Co. KG, Hamburg, Germany). Participants in fasting state (whose previous meal was at least 12 h before bronchoscopy) under continuous oxygen supplementation were consciously sedated with intravenous midazolam 0.05–0.1 mg·kg⁻¹ (154–307 nmol·kg⁻¹) just before, and during, the bronchoscopy. Lidocaine (10%) spray and lidocaine (2%) solution up to 8.2 mg·kg⁻¹ were used to anaesthetize the upper and lower airways. Participants were continuously monitored with a three-lead ECG, BP measurements, and oximetry during bronchoscopy and for 2 h thereafter. A lung function measurement was taken 4 h after the bronchoscopy and before discharge. Participants were examined by a physician before release and received the on-call physician’s emergency telephone number in case of medical problems.

During each bronchoscopy, two prespecified lung tissue sites (middle lobe and lingula) were sampled by bronchosorption; in addition, one bronchoalveolar lavage (BAL), two bronchial brushings, and two mucosal biopsies were taken in the following order: first bronchosorption, second bronchosorption, BAL, first bronchial brushing, second bronchial brushing, first biopsy, second biopsy.

2.4.2 Bronchosorption

Bronchosorption was performed to sample two lung tissue sites—the middle lobe and the lingula—during each bronchoscopy. The bronchosorption catheter (Mucosal Diagnostics, Midhurst, United Kingdom) was inserted through the working channel of the bronchoscope and directed to the middle lobe. Under visual guidance, the catheter tip was extruded and placed onto the bronchial mucosa for 30 s to absorb ELF. After closing the cover of the catheter tip and removing the bronchosorption catheter, the catheter tip was cut off and transferred into a pre-weighed cryotube and stored at −80°C until analysis. A second catheter was used with the same procedure to collect samples from the contralateral lung lobe of the lingula.

2.4.3 BAL

BAL was performed with 100 ml pre-warmed saline using standard techniques, as described previously (Atochina-Vasserman et al., 2011), in the middle lobe at the first bronchoscopy and in the lingula at the second bronchoscopy, or vice versa. Five 20 ml aliquots of 37°C sterile 0.9% saline were instilled, immediately aspirated and collected on ice into a graduated cylinder for documentation of recovered total volume. BAL was then filtered using a 100 μm mesh to remove debris and centrifuged at 4°C for 10 min at 300× g. BAL supernatant was removed, aliquoted and kept frozen at −80°C until analysis. The cell pellet was resuspended in 1 ml of ice-cold PBS. The total number of cells was determined after Trypan-blue staining using a Neubauer chamber. Cytospin slides were prepared using 20,000 cells by centrifugation at 23 × g for 3 min. After staining with Diff-Quik (RAL Diagnostics/Siemens Healthineers), cells were differentiated using light microscopy. The remaining cells were centrifuged, suspended in 1 ml RNAprotect (QIAGEN) and stored at −80°C until analysis.

BAL was initially sampled in duplicate from two contralateral lung segments, from each bronchoscopy in all participants from the pilot cohort and in the first three participants from the inhalation cohort. It was then decided to remove the duplicate BAL procedure to reduce the burden on the participants and to improve the ease of the procedures. Therefore, the majority of participants provided two BAL-obtained samples in total, but the first five participants provided four BAL-obtained samples.

2.4.4 Endobronchial forceps biopsy and brush biopsy

Two endobronchial biopsies taken using forceps (Olympus FB-231D, Olympus Europa SE & Co. KG, Hamburg, Germany) and two using bronchial brushing (Olympus cytology brush 5 mm, Olympus Europa SE & Co. KG, Hamburg, Germany) were obtained from different segments of the left lower lobe at the first bronchoscopy and in different segments of the right lower lobe at the second bronchoscopy, or vice versa. Mucosal biopsies were transferred from the forceps into pre-weighed cryotubes and stored at −80°C until analysis. Brush biopsies were cut and transferred into pre-weighed cryotubes and stored at −80°C until analysis. Samples from the first participants (all of the participants from the pilot cohort and three participants from the inhalation cohort) were eluted from the cut-off cytology brush by vortexing for 30 s in RNAprotect. They were then stored at −80°C until analysis.

2.4.5 Exhaled particles

Breath samples were collected for 15 min before bronchoscopy, using a commercially available device for particle sampling (Particles in Exhaled Air [PExA], Gothenburg, Sweden) according to the manufacturer’s instructions. Participants were asked to perform repetitive breathing manoeuvres that increased the number of collapsed airways and particle emission, as described previously (Larsson et al., 2012). For participants with low particle emission, sampling times were extended up to a maximum of 30 min with the aim of collecting about
240 ng of particle mass on the filter. The collection filters were stored at \(-80^\circ\)C until analysis.

2.4.6 | Blood sampling

Plasma samples for pharmacokinetic analysis were prepared from blood samples (2.6 mL) taken from the cubital vein immediately before, and 0.25, 0.5, 1, 2, 4, 8, 12 and 24 h after, drug administration. Blood was stored in ethylenediaminetetraacetic-acid-coated tubes and processed by centrifugation at 2000 \(\times\) g for 10 min at 4°C. Plasma was taken in one aliquot per blood sample and stored at \(-80^\circ\)C until analysis.

2.4.7 | Other clinical study procedures

BP and heart rate were determined from the left arm of participants in a seated position. Medical history, physical examination, ECG, height, weight, breath alcohol testing, safety blood sampling and processing, and urine sampling, including pregnancy testing, were also performed. Spirometry was measured according to current recommendations from the American Thoracic Society and the European Respiratory Society, using normal values according to the Global Lung Function Initiative (Quanjer et al., 2012).

2.5 | Study-stopping criteria

Continuation of the study was to be re-evaluated if any serious adverse event occurred, or any other event that, in the opinion of the investigator, posed undue risk to the study participants.

2.6 | Bioanalysis of lung and plasma samples

Quantification of drugs in all samples was performed using reverse-phase ultra-HPLC coupled with tandem mass spectrometer (LC–MS/MS) detection. Supernatant and cell pellets from the BAL procedure were analysed separately.

2.6.1 | Sample preparation

For plasma samples, an aliquot of 100 μl was protein precipitated with 360 μl methanol containing 0.2% acetic acid and stable-isotope-labelled internal standards for the compounds. After 1 min of mixing and centrifugation, 350 μl were transferred to a new plate and evaporated under a stream of nitrogen, as described above. The samples were redissolved in 125 μl 50% methanol containing 0.2% acetic acid.

For BAL fluid samples, an aliquot of 100 μl was diluted with 360 μl methanol containing 0.2% acetic acid and stable-isotope-labelled internal standards for the compounds. After mixing and centrifugation, 350 μl were transferred to a new plate and evaporated under a stream of nitrogen, as described above. The samples were redissolved in 125 μl 50% methanol containing 0.2% acetic acid.

For BAL cell pellet samples, for salbutamol, salmeterol and propranolol, an aliquot of 100 μl BAL cell suspension was diluted with 360 μl methanol containing 0.2% acetic acid and stable-isotope-labelled internal standards for the compounds. After mixing and centrifugation, 350 μl were transferred to a new plate and evaporated under a stream of nitrogen. The samples were redissolved in 125 μl 50% methanol containing 0.2% acetic acid. For fluticasone propionate, an aliquot of 50 μl BAL cell suspension was diluted with 50 μl 50 mM carbonate buffer (pH 10) and 5 μl methanol containing 0.2% acetic acid and stable-isotope-labelled internal standards for the compounds. After the addition of 600 μl methyl tert-butyl ether/isohexane (1:1), the samples were shaken for 20 min. The upper 550 μl organic layer was transferred to new tubes and evaporated under a stream of nitrogen. The samples were re-dissolved in 150 μl 50% methanol containing 0.2% acetic acid.

The weighed biopsies were homogenized using ceramic beads (Precellys, Bertin Instruments, Montigny-le-Bretonneux, France) in 200 μl isotonic Ringer’s solution. A 50 μl aliquot of the homogenate was protein precipitated with 180 μl methanol containing 0.2% acetic acid and stable-isotope-labelled internal standards for the compounds. After mixing and centrifugation, the supernatant was transferred to a new plate and diluted 1:1 with 0.2% acetic acid in water.

Drug concentrations in BAL fluid samples were corrected for dilution using the urea method (Rennard et al., 1986). An aliquot of 20 μl plasma or BAL fluid was diluted with 700 μl 90% acetonitrile containing stable-isotope-labelled internal standard (2N\(^{15}\)-urea). The plasma samples were diluted further by transferring 5 μl supernatant to a new plate and adding 600 μl 90% acetonitrile.

2.6.2 | Reversed-phase LC–MS/MS

Sample extracts were analysed on a SCIEX API 5000 triple quadrupole mass spectrometer with Turbo V Ion Source coupled with a SHIMADZU LC-20AD binary pump and a CTC HTC-xt autosampler. The sample extracts were injected onto a Phenomenex Kinetex C18 column (50 × 2.1 mm, 2.6 μm particle size). A short gradient (0.5 ml min\(^{-1}\) flow rate, 40°C column oven), using 10 mM ammonium acetate, 0.2% acetic acid in water (mobile phase A), and 0.2% acetic acid in methanol (mobile phase B), was applied. The gradient started at a plateau of 3% B for 0.5 min, increasing to 95% for 2.3 min and...
directly to a washing step at 95% B for 1 min, then returned to initial conditions followed by a 0.5-min column re-equilibration. MS data (multireaction monitoring [MRM]) were acquired in positive electrospray ionization mode, using the transitions 501.2 > 293.3, 504.2 > 293.3, 416.3 > 398.3, 419.3 > 401.3, 240.2 > 148.1, 243.2 > 151.1, and 260.2 > 183.3 for fluticasone propionate, fluticasone propionate-d3, salmeterol, salmeterol-d3, salbutamol, salbutamol-d3, and propranolol, respectively. For quantification of midazolam, with the transition 326.1 > 291.2, the mobile phase A was 0.2% formic acid in water, and the mobile phase B was 0.2% formic acid in acetonitrile with a gradient ranging from 5% B to 95% B for 1.5 min, followed by a washing step of 95% B for 1 min. De-clustering potential, mass energy, and exit potential were optimized for each individual compound. The temperature, curtain gas, and ion voltage were set to 550 °C, 15, and 5500 V, respectively. Data acquisition and quantification were performed using Analyst software version 1.6.3 (SCIEX). The lower limit of quantification for the compounds in all matrices is shown in Table S1. All analytical batches fulfilled the precision criteria of 85%–115% and a coefficient of variation of less than 15%.

For the urea method, quantification of urea was performed by hydrophilic interaction LC (HILIC) coupled with tandem MS. Sample extracts were analysed on a SCIEX API 5500 triple quadrupole mass spectrometer with Turbo V Ion Source coupled with an Agilent 1200 Series Binary Pump SL and a CTC HTC-xt autosampler. The sample extracts were injected onto an iHILIC-Fusion column (50 × 2.1 mm, 1.8 μm particle size) from HILICON. A gradient (0.5 ml·min⁻¹ flow rate, 40°C column oven), using purified water (mobile phase A) and acetonitrile (mobile phase B), was applied. The gradient started at 90% B, decreasing to 50% for 1 min, followed by a washing step at 50% B for 1.5 min, then returned to initial conditions followed by a 3-min column re-equilibration. MS data (MRM) were acquired in positive electrospray ionization mode, using the transitions 61 > 44 and 63 > 45 for urea and 2N15-urea (internal standard), respectively.

Quantification of drug concentrations in the BAL cell pellet and endobronchial brush-obtained samples was restricted because accurate weights of these samples were not available, because the sample volume was not obtained. Instead, concentrations of drugs in the processed samples for LC-MS/MS were determined. All samples were frozen directly after sampling and kept in frozen condition until analysis. After thawing, the remaining intact cells were completely lysed by adding organic solvent in excess. Concentrations of all inhaled drugs were determined together in one sample procedure, as were the oral drugs.

2.7 Data and statistical analysis

Being exploratory, this study had no formal primary or secondary objectives and thus no formal primary variable of interest; therefore, a formal sample size calculation was not performed. No previous data on ELF pharmacokinetics in samples obtained via bronchosorption or via exhaled particles were available. To test the hypothesis that the pharmacokinetics of selected drugs after inhalation or oral administration could be measured in lung samples of ELF, the collection of three data samples per time point (i.e. three biological replicates) was judged to be appropriate. The number of participants per group in this study was based on feasibility. Similar numbers are typically used in pilot and method development studies.

Descriptive statistics were generated for demographic, medication use during bronchoscopy, and BAL cell data for all cohorts, and for drug concentrations at all timepoints after dosing in the inhalation and oral cohorts, using R version 3.5.1 (R Project for Statistical Computing, RRID:SCR_001905) and RStudio version 1.1.463 (RStudio, RRID:SCR_000432), including the ggplot2 (ggplot2, RRID: SCR_014601) and Tidy package (tidyr, RRID:SCR_017102) (pilot cohort drug concentration data were not quantitative). Although patients were given midazolam twice during the study, once with the first bronchoscopy and again with the second, only concentrations in samples taken after the first bronchoscopy procedure are reported, because plasma and lung samples after the second bronchoscopy were not taken at matching time points. Lung:plasma ratios for each drug in bronchosorption-obtained ELF, BAL supernatant, and mucosal biopsy lung samples were computed as the ratio of the mean concentration in lung samples to the mean concentration in plasma, based on all observations for that drug. Pharmacokinetic profiles of each drug up to 18 h after administration were generated by combining data from multiple individuals, covering all sample timings. Systemic pharmacokinetic parameters of salbutamol (oral and inhaled) and propranolol—aera under the concentration–time curve, maximum observed concentration, time to maximum observed concentration and half-life—were estimated using non-compartmental analyses in R (version 3.5.1) and RStudio (version 1.1.463), including the PKNA package. The data and statistical analysis comply with the recommendations of the British Journal of Pharmacology on experimental design and analysis in pharmacology (Curtis et al., 2018).

2.8 Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in the IUPHAR/BPS Guide to PHARMACOLOGY (http://www.guidetopharmacology.org) and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20 (Alexander et al., 2019).

3 RESULTS

3.1 Participant disposition and characteristics

Overall, 26 healthy volunteers were screened for study participation: 20 were enrolled, and all completed the study (Figure 1). For one participant, the BAL procedure was not completed during either bronchoscopy. Details of minor protocol deviations are in the
supplementary material. The participants comprised 11 women and nine men, with a mean (SD) age of 30.8 (8.4) years and body mass index of 24.8 (3.0) kg/m². Their mean (SD) predicted FEV₁ was 99.6% (9.4%) and FEV₁ to FVC ratio was 79.6% (6.8%).

3.2 | Medication use during bronchoscopy

Use of midazolam and lidocaine for sedation and anaesthesia, respectively, during the bronchoscopies is detailed in Table S2. Mean (SD) midazolam use was 0.076 (0.013) mg kg⁻¹ during the first procedure and 0.077 (0.016) mg kg⁻¹ during the second. Mean (SD) lidocaine use was 6.86 (1.21) mg kg⁻¹ during the first procedure and 6.63 (1.37) mg kg⁻¹ during the second.

3.3 | BAL sample parameters

Mean (SD) BAL fluid recovery from the first bronchoscopy was 61.4 (5.6) ml. The distribution of cells in collected BAL fluid was as expected in healthy individuals (Table S3).

3.4 | Safety

Overall, the study procedures were well tolerated by study participants. In total, 31 adverse events in 13 participants were reported; all were mild (n = 17) to moderate (n = 14) in intensity and were resolved by study completion. The majority of adverse events were associated with bronchoscopy, with the most frequent being local irritation (sore throat, laryngeal pain, epistaxis; n = 6 total), fever (n = 4), or cough (n = 3) after bronchoscopy. There were no serious adverse events.

3.5 | Lung and plasma pharmacokinetics (inhalation and oral cohorts)

3.5.1 | Salbutamol

After inhalation, salbutamol concentrations were >100-fold higher in all lung samples than in plasma (Table 1). The pharmacokinetic profiles of salbutamol in the different lung samples were similar, with the greatest differences between drug concentrations in lung versus plasma being observed early after dosing; this then decreased over time (Figure 2a). The observed variability of salbutamol concentrations in ELF obtained via bronchosorption and BAL (supernatant) was comparable to the variability of concentrations in plasma (Figure 2c; Table S4).

Following oral administration, salbutamol concentrations in lung were in a similar range to systemic concentrations (Figure 2b), with a lung:plasma ratio of drug concentrations of ~1.0 (0.7–1.6; Table 1).

3.5.2 | Salmeterol

After inhalation of salmeterol, drug concentrations in lung samples were ~200–300-fold higher than systemic concentrations (Table 1). Similar pharmacokinetic profiles of salmeterol were obtained from the different lung samples (Figure 3a).

3.5.3 | Fluticasone propionate

After inhalation of fluticasone propionate, lung:plasma ratios were generally higher than those for salbutamol and salmeterol (Table 1). There were notable differences between the pharmacokinetic profiles from the different lung samples (Figure 3b). Concentrations in BAL supernatant were highest, at ~2000-fold higher than systemic concentrations. The concentrations in bronchosorption-obtained ELF were ~1000-fold higher than systemic concentrations. Quantifiable drug was present in only one of the mucosal biopsy samples.

3.5.4 | Propranolol

Orally administered propranolol resulted in high concentrations in plasma relative to the inhaled drugs. The drug concentrations in all lung samples were even higher than plasma (Figure 4), indicating extensive binding in tissue. Concentrations in mucosal biopsy samples and bronchosorption-obtained ELF were similar, but concentrations in ELF estimated from BAL supernatant were ~5-fold higher than those in bronchosorption-obtained samples (Table 1). Systemic pharmacokinetic parameters of propranolol are summarized in Table S5.

3.5.5 | Midazolam

Concentrations of midazolam in mucosal biopsy and plasma samples were similar after intravenous administration during the first bronchoscopy (Table 1, Figure 5). Concentrations in bronchosorption-obtained ELF were lower than in mucosal biopsy samples. As observed for propranolol, concentrations of midazolam in BAL supernatant were considerably (in this case, 40-fold) higher than in bronchosorption-obtained ELF.

3.6 | Particles in exhaled air (inhalation and oral cohorts)

Drug concentrations in the great majority of PEx samples were found to be below the lower limit of quantification. A
TABLE 1  Lung:plasma ratios and plasma protein binding of study drugs after inhaled, oral or intravenous administration

| Drug                     | Computed lung:plasma ratio\(^a\) for lung sample | Plasma protein binding, %\(^b\) |
|--------------------------|--------------------------------------------------|-------------------------------|
|                          | Bronchosorption  | BAL       | Mucosal biopsy |                      |
| Salbutamol (oral)        | 0.7              | 1.6       | 1.2            | 7–8\(^c\)            |
| Salbutamol (inhaled)     | 135              | 103       | 115            | 7–8\(^c\)            |
| Salmeterol (inhaled)     | 311              | 216       | 197            | 96                   |
| Fluticasone propionate (inhaled) | 939              | 2109      | 131            | 99                   |
| Propranolol (oral)       | 24               | 166       | 10             | ~90 (85–96)          |
| Midazolam (intravenous)  | 0.1              | 4.0       | 0.6            | > 95                 |

Abbreviation: BAL, bronchoalveolar lavage.
\(^a\)Lung:plasma ratios were computed as the ratio of the mean concentration in each lung sample type to the mean concentration in plasma, based on all observations for that drug.
\(^b\)Data from literature in PubChem database (Kim et al., 2019) unless noted otherwise.
\(^c\)Data from Morgan et al. (Morgan et al., 1986). No numerical data provided in PubChem.

FIGURE 2  Lung and systemic pharmacokinetic profiles of salbutamol from different sampling methods. (a) Inhaled salbutamol profiles (mean values) for bronchosorption, mucosal biopsy, BAL, and plasma samples. (b) Oral salbutamol profiles (mean values) for bronchosorption, mucosal biopsy, BAL and plasma samples. (c) Inhaled salbutamol individual-level data for bronchosorption, BAL, and plasma samples (each colour represents a different study participant, markers of the same colour indicate opposite lung sites of the same individual). See Table S4 for SDs and n numbers for panels a and b, and Table S1 for lower limit of quantification information. BAL, bronchoalveolar lavage

FIGURE 3  Lung and systemic pharmacokinetic profiles from different sampling methods after administration of (a) inhaled salmeterol and (b) inhaled fluticasone propionate. Graphs show mean values (see Table S4 for SDs and n numbers, and Table S1 for lower limit of quantification information). BAL, bronchoalveolar lavage
small number of samples had low and variable concentrations for salbutamol that were only slightly above the limit of quantification.

4 | DISCUSSION

The present study demonstrates the feasibility of obtaining pharmaco-kinetic profiles of drug concentrations in different compartments of the human lung. This was achieved by using a combination of established and novel sampling methods in an intensive sampling schedule to directly measure drug concentrations in different lung compartments. Lung targeting was evaluated for three inhaled drugs of different pharmacological classes, and that are commonly prescribed for respiratory disease, by comparing pulmonary drug concentrations with systemic concentrations, albeit quantification in terms of lung targeting of free drug is not established for available sampling methods. After inhalation of salbutamol, a short-acting $\beta_2$-adrenoceptor agonist, we observed drug concentrations in the bronchial mucosa and in the bronchial and peripheral ELF that were $>100$-fold higher than in plasma, whereas concentrations in lung and plasma were similar after oral administration. The concentration differences between lung and plasma after inhalation were even higher for salmeterol, a long-acting $\beta_2$-adrenoceptor agonist, and fluticasone propionate, an inhaled corticosteroid.

The different techniques used to obtain lung samples in this study had varying utility as tools for assessing pulmonary pharmacokinetics. Bronchosorption is a novel method developed for sampling bronchial ELF, which provided reproducible drug concentration data for both inhaled and orally administered drugs together with detailed associated information on the lung location from which they were obtained. This technique takes a pure sample of ELF from a known location in the central lung without diluting it, making it a sensitive and reliable method for quantifying the pulmonary pharmacokinetics of a drug. The utility of bronchosorption has been demonstrated previously for biomarker analysis (Leaker et al., 2015). Bronchosorption also caused less stress to participants than BAL, with the latter procedure being associated with cough and sometimes fever.

BAL is an established method for sampling ELF from the lung, but it is accompanied by several inherent challenges. First, BAL requires instillation of a relatively large volume of saline in the lung, resulting in dilution of ELF. Chemical analysis of this diluted ELF sample is challenging, requiring highly sensitive analyses and a correction method, such as the urea method (Rennard et al., 1986), to account for the dilution and to estimate drug concentrations in the BAL fluid. However, the urea correction method relies on the assumption that there is no extraction of urea nor of the drug analyte from surrounding lung tissue during the course of the BAL procedure (which takes $\sim2$–$3$ min) and that no urea leaks into the alveolar compartment with disease. In this context, the BAL urea dilution method is known to be affected in endotoxin-inflamed human lungs (Mole et al., 2020). Second, BAL is not spatially defined, so the sample collected is a mix of ELF from central and peripheral lung from the 4th to 5th generation down to the alveoli (20–23 generation). The differences observed between the drug concentrations in bronchosorption- versus BAL-obtained ELF are not unexpected because the samples are taken from different lung compartments. However, differences in methodology
between the two techniques, particularly the potential extraction of drug into the obtained ELF samples with BAL, may partly explain the higher concentrations we observed in BAL-obtained ELF compared with those in bronchosorption-obtained ELF (Forbes et al., 2011; Kelly et al., 1988).

Lung sampling via bronchoscopy was limited to two sessions of ~30 min each per study participant. Distributing the timing of the bronchoscopies and associated sampling procedures across each cohort and pooling the data for all participants allowed the generation of a pharmacokinetic time course of drug concentrations in lung for up to 18 h after dosing. The pharmacokinetic profiles derived from lung samples had a reasonably low level of variability, comparable with those from plasma samples. This study focused on generating replicate samples from the same site in the lung to give an understanding of variability and reproducibility rather than increasing sampling from different regions by the same technique. These results are important for designing future studies aiming at the generation of spatial lung pharmacokinetic data by mapping the airways with bronchosorption.

Concentrations of salbutamol in lung and plasma were similar after oral administration. This might be expected because low MW compounds are generally distributed homogenously throughout the body on a free drug basis, and additionally salbutamol does not bind extensively to either plasma proteins or tissue (Boulton & Fawcett, 2001; Morgan et al., 1986). However, after inhalation, salbutamol concentrations were ~100-fold higher in ELF and lung tissue than in plasma, with the greatest differences being observed early after dosing. These results provide a quantification of lung targeting for salbutamol after inhalation, which can be expected to translate to a therapeutic advantage of the inhaled dosage form. This therapeutic advantage will decrease as moving from central towards peripheral lung and from ELF towards blood through lung tissue as was recently described by PK/PD modelling (Boger & Fridén, 2019). This targeting is assumed to be primarily driven by the low permeability of salbutamol, which results in retention of the compound inside cells. Salmeterol is a more lipophilic basic compound than salbutamol that undergoes lysosomal trapping in lung tissue (Bäckström et al., 2016), which probably contributes to its pronounced lung retention. Consistently high concentrations of salbutamol and salmeterol in both bronchosorption- and BAL-obtained ELF may suggest that both drugs distribute to central and peripheral lung in similar manner. Fluticasone propionate demonstrated the greatest lung retention among the drugs studied, with determined concentrations in the ELF being up to ~2100-fold higher than in plasma after inhalation. Fluticasone is a drug with low solubility that is retained in the lung because of slow dissolution, limiting its absorption into the circulation (Johnson, 1996). BAL supernatants contained higher concentrations of fluticasone than bronchosorption-obtained samples. The difference may in part be attributed to the instilled saline during the BAL procedure, which probably recovers both drug in solution and undissolved particles, whereas bronchosorption probably limits samples to fluid. Interestingly, our qualitative data from the BAL cells indicated higher levels of fluticasone propionate compared with the relatively soluble drugs, such as salbutamol and salmeterol (Figure S1[a]). This may point to the role of alveolar macrophage phagocytosis for inhaled treatments with poorly soluble compounds.

For propranolol and midazolam, concentrations in ELF from BAL supernatant were ~7-fold and ~40-fold higher, respectively, than those in bronchosorption-obtained ELF. Interestingly, estimated concentrations in ELF from BAL supernatant were 166-fold and 4-fold higher than plasma for propranolol and midazolam, respectively, and higher still if one would account for free fraction in plasma for these drugs (~10% and <5%, respectively). This was unexpected because propranolol and midazolam are both permeable molecules and are not known to be substrates of any drug transporters. We conclude that this overestimation of concentrations in BAL-obtained ELF is due either to extraction of drug from the surrounding tissue during the BAL procedure or to recovery of the non-aqueous surfactant phase, which may contain high amounts of lipophilic (midazolam) or amphiphilic (propranolol) drug. Either way, this observation limits the general utility of BAL for drug quantification in ELF.

Drug concentrations in forceps-obtained mucosal tissue biopsies were similar to those in bronchosorption-obtained ELF, with the exception of fluticasone propionate, which unexpectedly was not quantifiable in most mucosal tissue samples. This finding is inconsistent with the high amounts of fluticasone propionate found in biopsy samples taken by bronchial brush and in airway cells from BAL cell pellet, and warrants further investigation.

We were not able to quantify drug concentrations in PEx samples in this study. The small absolute volume of ELF from small airways that was available from the maximal 15-min PEx collection periods resulted in drug concentrations that were below the detection limit of the LC-MS/MS assay. Pre-study estimation and data from the pilot cohort suggested that detection of drug in PEx could be possible, so this method was included in the protocol. However, post hoc calculations based on the highest actual concentrations we detected in bronchosorption- and BAL-obtained ELF indicated that, with the available sample preparation and analysis method, it would not have been possible to detect compounds in PEx samples. Although it is challenging to increase the PEx sampling time, improvements in pre-processing dilution steps and in the sensitivity of chemical analysis appear to be feasible, which may, in future, enable successful determination of non-volatile drug concentrations in PEx-obtained ELF.

Building on the findings of this study, we propose that simultaneous measurement of local pulmonary pharmacokinetics and pharmacodynamic biomarkers using bronchosorption may have great utility in early clinical drug development. This methodology would demonstrate local target engagement via correlation of pharmacodynamic biomarkers with drug pharmacokinetics in the same sample. This approach could not only demonstrate pharmacodynamic effect but also reveal whether the effect is locally or systemically driven and facilitate prediction of the dose regimen required to achieve effective drug levels in bronchial mucosa. The approach taken in this study represents a step towards the ultimate goal of quantifying free and pharmacologically active drug locally in the lung. Prospects for future work
in this direction include development of methods to determine the free fractions of drug in lung sample matrices and integrating all data into physiologically based pharmacokinetic frameworks to generate in silico predictions of free drug across all regions and structures of the lung.

In conclusion, this study demonstrated the lung targeting of three commonly prescribed inhaled drugs, which were shown to be present in considerably higher concentrations in bronchial mucosa and in bronchial and peripheral ELF than in plasma after inhalation. These findings demonstrate the feasibility and value of performing clinical pulmonary pharmacokinetic studies of novel lung-targeted drugs to describe target tissue concentrations and to support estimation of therapeutic dose and dosing interval.

ACKNOWLEDGEMENTS

We thank Trevor Hansel, MD, of Imperial College London (London, United Kingdom) for scientific exchange and technical advice. We thank the clinical study volunteers for their participation and the site staff for their diligent study conduct. We also thank Richard Claes, PhD, of PharmaGenesis London (London, United Kingdom) for providing medical writing support, which was funded by AstraZeneca (Gothenburg, Sweden) in accordance with Good Publication Practice 3 (GPP3) guidelines (http://www.ismpp.org/gpp3). This study was sponsored by AstraZeneca.

AUTHOR CONTRIBUTIONS

M.W.S., O.H., M.M., U.G.E., M.F., and J.M.H. contributed to the study design. M.W.S., O.H., B.D.E., C.F., M.M., P.B., M.F., S.S., A.J.L., and J.M.H. contributed to data acquisition. M.W.S., U.G.E., M.F., and J.M.-H. contributed to data analysis. All authors contributed to interpretation of results and drafting and critically reviewing the manuscript, and gave their final approval for submission.

DECLARATION OF TRANSPARENCY AND SCIENTIFIC RIGOUR

This Declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigour of preclinical research as stated in the BJP guidelines for Design and Analysis, and as recommended by funding agencies, publishers and other organisations engaged with supporting research.

CONFLICT OF INTERESTS

J.M.H.'s institution received funding from AstraZeneca for conducting the study. J.M.H. has received personal fees from Boehringer Ingelheim, HAL Allergy, Merck, and Novartis and grants to his institution from ALK, Allergopharma, Astellas, AstraZeneca. Boehringer Ingelheim, GlaxoSmithKline, Janssen Pharmaceutica, LETIPharma, Novartis, and Sanofi-Aventis outside the scope of this work. M.W.S., U.G.E., M.F., and A.J.L. are employees of AstraZeneca and may own stock or stock options. S.S. is a former employee of AstraZeneca and a current employee of Nordtec. O.H., B.D.E., C.F., M.M., and P.B. have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

This study is registered at ClinicalTrials.gov with identifier NCT03524066. Data underlying the findings described in this manuscript may be obtained in accordance with AstraZeneca's data sharing policy described at https://astrazenecagrouptrials.pharmacm.com/ST/Submission/Disclosure.

ORCID

Muhammad Waqas Sadiq https://orcid.org/0000-0002-6146-2971
Olaf Holz https://orcid.org/0000-0003-6665-7807
Philipp Badorrek https://orcid.org/0000-0003-2490-0583
Ulf G. Eriksson https://orcid.org/0000-0002-3504-3575
Jens M. Hohlfeld https://orcid.org/0000-0003-2646-6186

REFERENCES

Alexander, S. P. H., Christopoulos, A., Davenport, A. P., Kelly, E., Mathie, A., Peters, J. A., Veale, E. L., Armstrong, J. F., Facenda, E., Harding, S. D., Pawson, A. J., Sharman, J. L., Southan, C., Davies, J. A., & CGTP Collaborators. (2019). The Concise Guide to PHARMACOLOGY 2019/20: G protein-coupled receptors. British Journal of Pharmacology, 176, 521–5141. https://doi.org/10.1111/bjp.14748
Atochina-Vasserman, E. N., Winkler, C., Abramova, H., Schaumann, F., Krug, N., Gow, A. J., Beers, M. F., & Hohlfeld, J. M. (2011). Segmental allergen challenge alters multimeric structure and function of surfactant protein D in humans. American Journal of Respiratory and Critical Care Medicine, 183, 856–864. https://doi.org/10.1164/rccm.201004-0654OC
Bäckström, E., Boger, E., Lundqvist, A., Hammarlund-Udenaes, M., & Fridén, M. (2016). Lung retention by lysosomal trapping of inhaled drugs can be predicted in vitro with lung slices. Journal of Pharmaceutical Sciences, 105, 3432–3439. https://doi.org/10.1016/j.xphs.2016.08.014
Bake, B., Larsson, P., Ljungkvist, G., Ljungström, E., & Olin, A.-C. (2019). Exhaled particles and small airways. Respiratory Research, 20, 8. https://doi.org/10.1186/s12931-019-0970-9
Bleecker, E. R., McFadden, E. R., Boushey, H. A., Edell, E. S., Eschenbacher, W. L., Godard, P. G., Goldstein, R. A., Holgate, S. T., Hunninghake, G. W., Hurd, S. S., Laitinen, A., Lichtenstein, L. M., Prograis, L., Rankin, J. A., Ram, J. S., Reed, C. E., Reynolds, H. Y., & Wood, R. E. (1991). Workshop summary and guidelines: Investigative use of bronchoscopy, lavage, and bronchial biopsies in asthma and other airway diseases. The Journal of Allergy and Clinical Immunology, 88, 808–814.
Boger, E., & Fridén, M. (2019). Physiologically based pharmacokinetic/pharmacodynamic modeling accurately predicts the better bronchodilatory effect of inhaled versus oral salbutamol dosage forms. Journal of Aerosol Medicine and Pulmonary Drug Delivery, 32, 1–12. https://doi.org/10.1089/jamp.2017.1436
Borghardt, J. M., Klaft, C., & Sharma, A. (2018). Inhaled therapy in respiratory disease: The complex interplay of pulmonary kinetic processes. Canadian Respiratory Journal, 2018, 2732017.
Boulton, D. W., & Fawcett, J. P. (2001). The pharmacokinetics of levosalbutamol: What are the clinical implications? Clinical Pharmacokinetics, 40, 23–40. https://doi.org/10.2165/00003088-200140010-00003
British Thoracic Society Bronchoscopy Guidelines Committee. (2001). British Thoracic Society guidelines on diagnostic flexible bronchoscopy. Thorax, 56(Suppl 1), 11–21.
Curtis, M. J., Alexander, S., Cirino, G., Docherty, J. R., George, C. H., Giembycz, M. A., Hoyer, D., Insel, P. A., Izzo, A. A., Ji, Y.,
SADIQ ET AL.

The effects of TRPV4 inhibition on pulmonary-vascular barrier permeability following segmental endotoxin challenge. Pulmonary Pharmacology & Therapeutics, 64, 101977. https://doi.org/10.1016/j.pulph.2020.101977

Morgan, D. J., Paull, J. D., Richmond, B. H., Wilson-Envered, E., & Ziccone, S. P. (1986). Pharmacokinetics of intravenous and oral salbutamol and its sulphate conjugate. British Journal of Clinical Pharmacology, 22, 587–593. https://doi.org/10.1111/j.1365-2125.1986.tb02939.x

Quanjer, P. H., Stanojevic, S., Cole, T. J., Baur, X., Hall, G. L., Culver, B. H., Enright, P. L., Hankinson, J. L., Ip, M. S. M., Zheng, J., Stocks, J., & the ERS Global Lung Function Initiative. (2012). Multi-ethnic reference values for spirometry for the 3–95-yr age range: The Global Lung Function 2012 equations. The European Respiratory Journal, 40, 1324–1343. https://doi.org/10.1183/09031936.00080312

Rennard, S. I., Basset, G., Lecossier, D., O'Donnell, K. M., Pinkston, P., Martin, P. G., & Crystal, R. G. (1986). Estimation of volume of epithelial lining fluid recovered by lavage using urea as marker of dilution. Journal of Applied Physiology (1985), 60, 532–538. https://doi.org/10.1152/jappl.1986.60.2.532

Schwarz, K., Biller, H., Windt, H., Koch, W., & Hohlfeld, J. M. (2010). Characterization of exhaled particles from the healthy human lung—A systematic analysis in relation to pulmonary function variables. Journal of Aerosol Medicine and Pulmonary Drug Delivery, 23, 371–379. https://doi.org/10.1089/jamp.2009.0809

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Sadiq, M. W., Holz, O., Ellingshausen, B. D., Faulenbach, C., Müller, M., Badorrek, P., Eriksson, U. G., Fridén, M., Storimlov, S., Lundqvist, A. J., & Hohlfeld, J. M. (2021). Lung pharmacokinetics of inhaled and systemic drugs: A clinical evaluation. British Journal of Pharmacology, 178(22), 4440–4451. https://doi.org/10.1111/bph.15621