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Simultaneous assessment of the pharmacokinetics of a pleuromutilin, lefamulin, in plasma, soft tissues and pulmonary epithelial lining fluid

M. Zeitlinger[1]*†, R. Schwameis[1]†, A. Burian[1], B. Burian[1], P. Matzneller[1], M. Müller[1], W. W. Wicha[2], D. B. Strickmann[2] and W. Prince[2]

[1]*Department of Clinical Pharmacology, Medical University of Vienna, Währinger Gürtel 18-20, 1090 Vienna, Austria;*
[2]*Nabriva Therapeutics AG, Leberstrasse 20, 1110 Vienna, Austria*

*Corresponding author. Tel: +43-1-40400-29890; Fax: +43-1-40400-29990; E-mail: markus.zeitlinger@meduniwien.ac.at
†Contributed equally to the work.

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Background: Lefamulin is a pleuromutilin antibiotic under evaluation for the treatment of bacterial infections, including respiratory tract infections. Currently, there are no high-quality pharmacokinetic data on drug tissue concentrations of lefamulin available.

Methods: A single dose of intravenous lefamulin (150 mg) was given to 12 healthy men. The registered EudraCT number for this study was 2010-021938-54. Lefamulin concentrations were simultaneously measured in plasma, skeletal muscle tissue, subcutaneous adipose tissue and epithelial lining fluid (ELF) over 24 h, and corresponding pharmacokinetic parameters were calculated. Microdialysis was used to measure unbound lefamulin concentrations in skeletal muscle tissue and subcutaneous adipose tissue, which were similar to unbound lefamulin concentrations in plasma. Bronchoalveolar lavage was performed 1, 2, 4 and 8 h post-dose to determine lefamulin concentrations in ELF.

Results: Unbound lefamulin levels showed a 5.7-fold higher exposure in ELF compared with that in plasma, demonstrating good penetration to the target site.

Conclusions: Lefamulin may be an addition to the therapeutic armamentarium for the treatment of infections. Simultaneous measurements of unbound drug concentration can guide target attainment for future therapeutic trials.

Introduction

The ongoing emergence of bacteria resistant to registered antibiotics has necessitated the development of novel antimicrobial substances with unique mechanisms of action.[1–3] Pleuromutilins are a group of antibiotics that inhibit bacterial protein synthesis by interacting with the peptidyl transferase centre of the 50S subunit of prokaryotic ribosomes.[4] The interaction of pleuromutilin antibiotics with the central part of domain V of the 23S ribosomal RNA is different from interactions of other antibiotics and is followed by an incorrect positioning of the CCA ends of transfer RNA for peptide transfer.[5] Hence, cross-resistance with other prokaryotic protein synthesis inhibitors is unlikely.[4] Lefamulin (BC-3781), a novel semi-synthetic pleuromutilin, is the first drug of its class to be developed for systemic application in humans. Lefamulin shows excellent activity against Gram-positive bacteria, including MDR *Streptococcus pneumoniae*, MRSA, *Moraxella catarrhalis* and *Haemophilus influenzae*, as well as atypical pathogens, including *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae* and *Legionella pneumophila*.[6,7] Besides lefamulin's novel antimicrobial mechanism of action and advantageous pharmacokinetics in humans,[8] it has high oral bioavailability, so intravenous and oral formulations are being developed.

Recently, a Phase 2 study in patients with acute bacterial skin and skin structure infections (ABSSSIs) demonstrated that lefamulin was well tolerated and produced clinical responses similar to those of the comparator, vancomycin.[9] Lefamulin is also under development for the treatment of respiratory tract infections (RTIs). Consequently, adequate lefamulin exposure levels in soft tissues and in epithelial lining fluid (ELF) are of major importance, and assessments of the appropriateness of intended dosing regimens would help predict the efficacy of lefamulin in RTIs and ABSSSIs. Measuring drug tissue concentration in different compartments could require a number of time-consuming studies. Therefore, a study design was developed for measuring drug concentrations at multiple target sites in the same subjects to obtain simultaneous pharmacokinetic data.

In this study, lefamulin concentrations were measured in plasma, skeletal muscle tissue, subcutaneous adipose tissue and ELF of healthy male volunteers.

Methods

Study design and study population

This open-labelled, non-randomized, single-centre Phase 1 pharmacokinetic study was performed at the Medical University of Vienna in accordance with the Declaration of Helsinki and the International Conference of Harmonization on Good Clinical Practice. The ethics committee of the Medical University of Vienna approved the protocol and the informed consent form. All subjects provided written informed consent before inclusion in the study. Eligible subjects were healthy men between 18 and 55 years old with a BMI in the range of 19–28 kg/m^2 and had no relevant medical history. Twelve subjects were screened and, after having satisfied the inclusion and exclusion criteria, were included in the study. The registered EudraCT number for this study was 2010-021938-54.

Study medication

Lefamulin (150 mg), supplied by Nabriva Therapeutics AG, was infused as prepared in 400 mL of 0.9% saline. All subjects were dosed intravenously with 150 mg of lefamulin, infused over 1 h in the morning.

Study procedures

Subjects arrived at the investigators' site at ~20:00 h on the day before dosing and were given a standard dinner. Subjects then fasted overnight until midday or 2 h after a bronchoalveolar lavage (BAL) procedure, whichever was longer. Subjects were released from the ward at the discretion of the investigator ~24 h after the administration of lefamulin. Subjects attended the site within 7–10 days for a follow-up visit.

Plasma samples were collected from a venous catheter inserted into the arm at the start of dosing and 0.5, 1, 1.25, 1.5, 2, 3, 4, 6, 8, 12, 16 and 24 h after the start of infusion.

Microdialysis (MD) samples of the interstitial space fluid in skeletal muscle tissue and subcutaneous adipose tissue were collected using probes (63 MD Catheter, μdialysis, Sweden). The probes were placed into the subcutaneous adipose tissue and in the *musculus quadriceps femoris* of the same thigh pre-dose, and samples were collected 0–1, 1–1.5, 1.5–2, 2–2.5, 2.5–3, 3–4, 5–6, 7–8, 11–12, 15–16 and 23–24 h after the start of infusion. At the end of the sampling period, probes were calibrated by retrodialysis before removal.

ELF samples were taken using BAL at 1 h, 2 h, 4 h or 8 h after the start of infusion. BAL was performed according to a standard operating procedure guideline of our institution. Because of the invasiveness of the procedure, each volunteer underwent one BAL procedure, and timepoints were randomized between the subjects in order to obtain three samples per timepoint. During the BAL procedure, 2% lidocaine spray, gel and solution were used for local anaesthesia of the pharynx, the nasal passages and the larynx, respectively. Also, subjects were sedated with 2–10 mg of midazolam intravenously immediately before BAL was performed. During the procedure, vital signs, ECG, frequent blood pressure and oxygen saturation were monitored. Oxygen was delivered by nasal cannula at a rate of up to 10 L/min as needed to maintain adequate oxygenation (oxygen saturation >90%). A bronchoscope (Olympus BF-P20D Flexible Bronchoscope, Olympus, Japan) was inserted through the nose and guided into the right bronchus, and after inspection of the bronchus, the bronchoscope was wedged either at segments B4–B5 of the right middle lobe or to B9 of the right lower lobe. Afterwards, the segment was rinsed three times with 20 mL of saline that was subsequently retrieved gently after each aliquot by suction. The aliquots were prepared for analysis and snap frozen at −80°C.

Drug concentration assessments

Microdialysis sampling

Lefamulin concentrations were evaluated in skeletal muscle tissue and subcutaneous adipose tissue using MD sampling. This method is based on the exchange of molecules between the extracellular space of the investigated tissue and an MD probe implanted into this tissue.[10,11] Solely free, i.e. non-protein-bound, molecules, which can diffuse across the membrane at the tip of the MD probe, were collected for subsequent analysis.

The diffusion process across the semipermeable membrane is quantitatively equal in both directions. The retrodialysis method relies on this principle, which implies that the fraction of the interstitial drug concentration that is recovered in the collected microdialysate sample, and is referred to as relative (*in vivo*) recovery, can be calculated by using the 'loss rate' observed during retrodialysis by the following equation:

$$\text{Relative } (\textit{in vivo}) \text{ recovery } (\%) = 100 - \left(100 \times \frac{\text{analyte concentration}_{\text{out}}}{\text{analyte concentration}_{\text{in}}}\right).$$

Interstitial lefamulin concentrations were calculated, as previously described,[11] as follows:

$$\text{Interstitial concentration} = 100 \times \left[\frac{\text{sample concentration}}{\text{relative } (\textit{in vivo}) \text{ recovery}}\right].$$

BAL fluid sampling

BAL relies on the concept that by rinsing the bronchoalveolar tree with saline, ELF can be collected. Since each BAL sample consisted of ELF in saline, the dilution of ELF was determined to translate drug concentrations in BAL fluid to drug concentrations in ELF. The urea dilution method was used to assess ELF dilution. Urea freely diffuses through several body compartments, including ELF. If the concentrations of urea in plasma and BAL are known, the volume of recovered ELF can be calculated and the concentration of lefamulin in ELF determined.[12] Concentrations (conc) of lefamulin in BAL samples were corrected for the procedure related dilution of ELF by saline as follows:

$$\text{Conc(lefamulin in ELF)}_{\text{corr}} = \frac{\text{conc(lefamulin in BAL)}_{\text{measured}}}{[\text{conc(urea in BAL)/conc(urea in plasma)}]}$$

Pharmacokinetic analysis

Pharmacokinetic parameters were calculated using a computer software package (SAS 9.1.3; SAS Institute, USA) in a Windows XP environment. The values C_{max}, T_{max}, terminal elimination $t_{1/2}$, AUC_{0-8} and AUC_{0-12} were calculated from non-fitted data by employing the trapezoidal rule. For $AUC_{0-\infty}$, individual extrapolation based on the last observed concentration and the elimination constant k_{el} was performed. Additionally, apparent total body CL and apparent V of distribution were calculated.

In the interstitial space fluid of skeletal muscle and subcutaneous adipose tissue and in ELF, T_{max}, C_{max} and AUC values were calculated from non-fitted data. To determine AUC_{0-12} in ELF, the lefamulin concentration after 12 h (C_{12}) was calculated using the formula $C = C_0 \cdot e^{kel \cdot t}$, where C represents the concentration at a defined timepoint, C_0 is the last concentration measured, k_{el} is the elimination rate constant and t is the time between the measurement of C_0 and the defined timepoint.

To obtain a conservative estimation of AUC_{0-24} after twice-daily application of lefamulin, the respective AUC_{0-12} values for plasma, skeletal muscle tissue, adipose tissue and ELF were doubled.

Bioanalysis

Plasma samples were analysed for lefamulin concentration using a previously validated LC-MS/MS method at A & M, Bergheim, Germany. MD and BAL samples were quantified by using a partially validated LC-MS/MS method for lefamulin in saline in the same laboratory. The lower limit of quantification for lefamulin was 1 ng/mL in both methods.

Results

All 12 volunteers who were screened enrolled in and completed the study. The study population was of white origin with a mean age of 26.9 years (SD 8.1 years), height of 183.3 cm (SD 7.8 cm), weight of 77.6 kg (SD 12.2 kg) and BMI of 23.0 kg/m^2 (SD 2.5 kg/m^2).

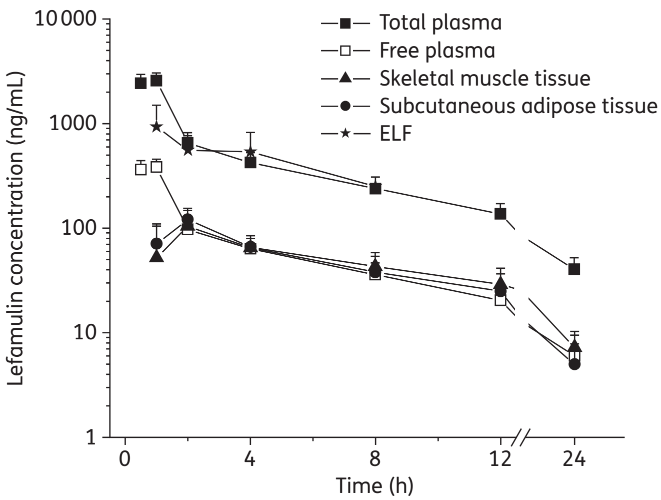

Figure 1. Mean±standard deviation concentration–time curves of lefamulin in plasma (n=12), skeletal muscle tissue (n=10), subcutaneous adipose tissue (n=8) and ELF (n=3) after intravenous administration of 150 mg of lefamulin over 1 h. For free plasma concentrations, a protein-unbound fraction of 13% was estimated and used for calculations.

Concentration–time curves of lefamulin in plasma, skeletal muscle tissue, adipose tissue and ELF are shown in Figure 1. The pharmacokinetic parameters of lefamulin in plasma, skeletal muscle tissue, adipose tissue and ELF are summarized in Table 1. Ratios of lefamulin tissue concentrations compared with corresponding free plasma concentrations are presented in Figure 2. Equilibration between protein-unbound lefamulin in plasma and skeletal tissue and subcutaneous tissues took ∼2 h.

In plasma, the median total AUC_{0-8} was 4985 ng·h/mL, the mean $AUC_{0-\infty}$was 7149 ng·h/mL and the median C_{max} was 2539 ng/mL. T_{max} occurred between 0.5 and 1 h after the start of infusion, with a median of 1 h (end of the infusion). The median terminal $t_{1/2}$ was 7.0 h. In ELF, the mean total AUC_{0-8} was 3871 ng·h/mL, median C_{max} was 706 ng/mL and, similar to that of plasma, T_{max} occurred at 1 h. The individual lefamulin concentration levels in ELF approached equilibrium with total plasma levels ∼2 h after the start of infusion. Accumulation ratios demonstrated that exposure to lefamulin in ELF was 75% of total plasma (accumulation ratio for AUC_{0-8} of 0.75) following a single intravenous dose of 150 mg. As only unbound drug can pass through the cell membrane into the ELF, the lefamulin concentrations in ELF were compared with unbound lefamulin in plasma. Exposure to lefamulin in ELF was ∼5.7-fold higher than the unbound fraction in plasma.

Median values of pharmacokinetic parameters in the interstitial space of skeletal muscle and subcutaneous adipose tissues were similar: mean AUC_{0-24} values were 870.0 ng·h/mL and 839.0 ng·h/mL, respectively. In skeletal muscle tissue and subcutaneous adipose tissue, the median C_{max} was 138.1 ng/mL and 145.1 ng/mL, respectively. T_{max} in skeletal muscle tissue and subcutaneous adipose tissue was 1.5 h and 1.25 h, respectively.

Accumulation ratios in skeletal and adipose tissue were calculated using the free fraction of lefamulin in plasma as reference. Exposure in tissues was similar to plasma exposure (98% and 87% for AUC_{0-24}). The results indicate fast exchange of lefamulin within the central compartment and interstitium. Free lefamulin concentration–time profiles in tissue and plasma were similar.

Table 1. Summary of the main pharmacokinetic parameters of 150 mg of lefamulin administered as a 1 h infusion (corrected for recovery where appropriate)

	Plasma, n=12	Free plasma[a], n=12	Skeletal muscle tissue, n=10	Subcutaneous adipose tissue, n=8	ELF[b], n=3
AUC_{0-8} (ng·h/mL)	4985 (3704–7866)	648.1 (481.5–1022.6)	496.3 (265–850)	604.9 (328–764)	3871
AUC_{0-12} (ng·h/mL)	5772 (4213–9014)	750.4 (547.7–1171.8)	632.1 (385–1047)	738.3 (405–879)	4489[c]
C_{max} (ng/mL)	2539 (1834–3458)	330.1 (238.4–449.5)	138.1 (51–466)	145.1 (81–551)	706
T_{max} (h)	1.00 (0.50–1.00)		1.50 (1.25–3.50)	1.25 (1.25–2.25)	1.00
$t_{1/2}$ (h)	6.866 (5.83–9.51)		—	—	—
CL (mL/min)	350.7 (228–474)		—	—	—
V (L)	202.8 (134–376)		—	—	—
AUC_{0-24} (ng·h/mL)	11554 (8426–18024)[d]	1500.8 (1095–2343)[d]	1264.2 (770–2094)[d]	1456.6 (810–1758)[d]	8978[d]

Values are given as median (range).
[a]Free plasma concentrations were calculated assuming plasma protein binding of 87%.
[b]Derived from the mean concentrations of the three subjects per timepoint.
[c]C_{12} was calculated by extrapolation and AUC_{0-12} was derived; each subject underwent only one BAL, and therefore no range is presented for ELF.
[d]Values calculated from corresponding AUC_{0-12}.

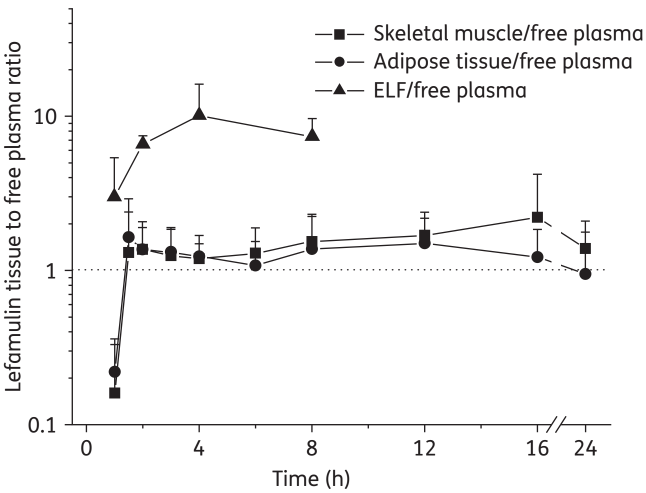

Figure 2. Ratios of lefamulin concentrations in skeletal muscle tissue, adipose tissue and ELF compared with free plasma concentrations over time. Values >1 describe concentrations exceeding free lefamulin concentrations achieved in plasma.

In general, 150 mg of lefamulin administered as a 1 h infusion was well tolerated. Local tolerability of lefamulin was assessed 1, 2 and 3 h after the start of the infusion. No local adverse event was observed for any of the 12 subjects. No serious adverse event was reported, and no subject discontinued the study because of adverse events. Seven subjects reported adverse events; 13 events were of mild intensity and 7 were of moderate intensity. Four subjects experienced 10 adverse events that were considered possibly or probably related to lefamulin administration. These included headache (reported by three subjects), increased serum bilirubin levels (in two subjects) and increased body temperature, diarrhoea, malaise, chills and local infusion-site pain (each in one subject). However, three subjects showed a combination of influenza-like symptoms (increased body temperature, increased bilirubin, malaise, chills, mild leucocytosis, headache) that accounted for the majority of possibly related adverse events. In all three cases, the adverse events occurred after bronchoscopy, and influenza-like symptoms have been previously described as a side effect of the BAL procedure.[13,14]

Discussion

The primary objective of this study was to determine and compare the concentration of lefamulin in plasma, skeletal muscle tissue, subcutaneous adipose tissue and ELF of lungs. After a single 150 mg intravenous infusion of 1 h, lefamulin showed rapid penetration into the interstitial space of skeletal muscle, subcutaneous adipose tissue and ELF. As MD measures only the free, i.e. protein-unbound, concentration of drug and therefore the fraction of a drug available for microbiological activity, the tissue exposure levels in interstitial space fluid of subcutaneous adipose and skeletal muscle tissue were comparable to those obtained in plasma ($f\mathrm{AUC}_{0-24}$) of the same subjects. The results indicate that unbound lefamulin freely diffuses into interstitial fluids of skeletal muscle tissue and adipose tissue.

Exposure levels in ELF were 5.7-fold higher than the free fraction in plasma ($f\mathrm{AUC}_{0-8}$). Similar accumulation effects in ELF are known for other antibiotics: e.g. macrolides and quinolones, which are used as treatments for RTIs.[15,16] Beside other factors, high concentrations of macrolides have been attributed to the active transport of the antibiotic from the plasma to the ELF by P-glycoprotein.[17] Since lefamulin is also a substrate of P-glycoprotein, it might likewise be actively transported into the ELF. Macrolides and fluoroquinolones are in addition known to accumulate in alveolar macrophages.[18] Alveolar macrophages might be disrupted during the BAL procedure, thereby releasing intracellular compounds. However, by processing BAL samples immediately under cooled conditions, every effort was taken to avoid this potential artefact in the present study. In addition, it should be noted that, in contrast to MD results, total lefamulin concentrations in ELF were determined in samples that were obtained by BAL. Lung surfactant contains little protein to which lefamulin might bind, and binding was not specifically assessed in the present study. Within the 12 h dosing interval, elimination of lefamulin from ELF appeared similar to that from plasma. After lefamulin infusion, equilibration of unbound drug between the central compartment and soft tissues was fast resulting in similar concentrations in plasma, skeletal and subcutaneous tissues. The rapid drug penetration into the investigated tissues after a single therapeutic dose of lefamulin supports its development for the use in indications such as RTI and ABSSSI. Interestingly, penetration of lefamulin into ELF was faster and more extensive than that into skeletal and subcutaneous tissues and resulted in ELF concentrations corresponding to total plasma concentrations. This effect indicates that an active transport mechanism is involved in the secretion of lefamulin from plasma to ELF.

To our knowledge, this is the first study that combined MD and BAL to determine concentrations of an antibiotic in the interstitial space fluid of peripheral soft tissue and in ELF, simultaneously, in humans. Simultaneous measurement of tissue concentrations in various compartments in the same subject provided a useful overview of the disposition of lefamulin within the body. Pharmacokinetic information from target tissue is a useful indicator for target attainment analysis to determine the possible therapeutic doses for future clinical trials.

Combining techniques has the potential to reduce both time and consequently costs in early-phase clinical drug development, although measurements of multiple compartments, by use of different techniques in one study, does increase single-study costs. Besides costs, ethical considerations should be taken into account. Incorporating multiple measures into one study will reduce the numbers of healthy volunteers that have to be exposed to a novel study drug without any possibility of personal benefit. By reducing the time of development, faster proceeding into a setting where patients might have personal benefit from a therapy will further improve the overall risk–benefit balance of a drug development programme.

However, the intrinsic limitations of this study have to be considered. Skeletal muscle and adipose tissue data were available for 10 and 8 subjects, respectively, due to MD probe failure. Further, the use of BAL to obtain samples for determination of ELF concentrations has limitations in terms of temporal resolution and the disputed urea dilution method for ELF concentration calculation.[19,20] In addition, it has to be considered that performing only one study instead of two has the disadvantage that, overall, less information on plasma pharmacokinetics, safety and tolerability is collected, just because of the fact that fewer subjects are exposed to the study drug. However, the advantages of combining different methods to simultaneously measure antibiotic concentrations in multiple tissues are of major interest in the field of early clinical drug development.

This study demonstrated good and rapid penetration of lefamulin into skeletal muscle and adipose tissue while showing extensive penetration and accumulation in ELF, even though concentrations were measured after a single dose. Hence, lefamulin may be a reasonable addition to the armamentarium of therapeutics for the treatment of RTIs and ABSSSIs, and it warrants further investigation.

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Transparency declarations

M. Z., R. S., A. B., B. B., P. M. and M. M. are or were employees of the Medical University of Vienna, which received grant support for the conduct of the study from Nabriva Therapeutics AG. W. W. W., D. B. S. and W. P. are employees of Nabriva Therapeutics AG.

The sponsor had no influence on the clinical conduct of the study, the data collection and the analysis of the samples.

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References

1 Hawkey PM, Jones AM. The changing epidemiology of resistance. *J Antimicrob Chemother* 2009; **64** Suppl 1: i3–10.

2 Canton R, Morosini MI. Emergence and spread of antibiotic resistance following exposure to antibiotics. *FEMS Microbiol Rev* 2011; **35**: 977–91.

3 Boucher HW, Talbot GH, Bradley JS *et al*. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. *Clin Infect Dis* 2009; **48**: 1–12.

4 Long KS, Hansen LH, Jakobsen L *et al*. Interaction of pleuromutilin derivatives with the ribosomal peptidyl transferase center. *Antimicrob Agents Chemother* 2006; **50**: 1458–62.

5 Davidovich C, Bashan A, Auerbach-Nevo T *et al*. Induced-fit tightens pleuromutilins binding to ribosomes and remote interactions enable their selectivity. *Proc Natl Acad Sci USA* 2007; **104**: 4291–6.

6 Sader HS, Paukner S, Ivezic-Schoenfeld Z *et al*. Antimicrobial activity of the novel pleuromutilin antibiotic BC-3781 against organisms responsible for community-acquired respiratory tract infections (CARTIs). *J Antimicrob Chemother* 2012; **67**: 1170–5.

7 Sader HS, Biedenbach DJ, Paukner S *et al*. Antimicrobial activity of the investigational pleuromutilin compound BC-3781 tested against Gram-positive organisms commonly associated with acute bacterial skin and skin structure infections. *Antimicrob Agents Chemother* 2012; **56**: 1619–23.

8 Rubino CM, Xue B, Bhavnani SM *et al*. Population pharmacokinetic analyses for BC-3781 using Phase 2 data from patients with acute bacterial skin and skin structure infections. *Antimicrob Agents Chemother* 2015; **59**: 282–8.

9 Prince WT, Ivezic-Schoenfeld Z, Lell C *et al*. Phase II clinical study of BC-3781, a pleuromutilin antibiotic, in treatment of patients with acute bacterial skin and skin structure infections. *Antimicrob Agents Chemother* 2013; **57**: 2087–94.

10 de la Pena A, Liu P, Derendorf H. Microdialysis in peripheral tissues. *Adv Drug Delivery Rev* 2000; **45**: 189–216.

11 Muller M. Science, medicine, and the future: microdialysis. *BMJ* 2002; **324**: 588–91.

12 Rennard SI, Basset G, Lecossier D *et al*. Estimation of volume of epithelial lining fluid recovered by lavage using urea as marker of dilution. *J Applied Physiol* 1986; **60**: 532–8.

13 Um SW, Choi CM, Lee CT *et al*. Prospective analysis of clinical characteristics and risk factors of postbronchoscopy fever. *Chest* 2004; **125**: 945–52.

14 Burns DM, Shure D, Francoz R *et al*. The physiologic consequences of saline lobar lavage in healthy human adults. *Am Rev Respir Dis* 1983; **127**: 695–701.

15 Kikuchi E, Yamazaki K, Kikuchi J *et al*. Pharmacokinetics of clarithromycin in bronchial epithelial lining fluid. *Respirology* 2008; **13**: 221–6.

16 Capitano B, Mattoes HM, Shore E *et al*. Steady-state intrapulmonary concentrations of moxifloxacin, levofloxacin, and azithromycin in older adults. *Chest* 2004; **125**: 965–73.

17 Togami K, Chono S, Morimoto K. Transport characteristics of clarithromycin, azithromycin and telithromycin, antibiotics applied for treatment of respiratory infections, in Calu-3 cell monolayers as model lung epithelial cells. *Die Pharmazie* 2012; **67**: 389–93.

18 Honeybourne D. Antibiotic penetration in the respiratory tract and implications for the selection of antimicrobial therapy. *Curr Opin Pulm Med* 1997; **3**: 170–4.

19 Kiem S, Schentaεg JJ. Interpretation of antibiotic concentration ratios measured in epithelial lining fluid. *Antimicrob Agents Chemother* 2008; **52**: 24–36.

20 Dahyot C, Marchand S, Pessini GL *et al*. Microdialysis study of imipenem distribution in skeletal muscle and lung extracellular fluids of *Acinetobacter baumannii*-infected rats. *Antimicrob Agents Chemother* 2006; **50**: 2265–7.