Eur Respir J
1992, 5, 471–476

The distribution of temafloxacin in bronchial epithelial lining fluid, alveolar macrophages and bronchial mucosa

D.R. Baldwin*, R. Wise**, J.M. Andrews**, J.P. Ashby**, D. Honeybourne*

The distribution of temafloxacin in bronchial epithelial lining fluid, alveolar macrophages and bronchial mucosa. D.R. Baldwin, R. Wise, J.M. Andrews, J.P. Ashby, D. Honeybourne.

ABSTRACT: The concentrations of temafloxacin, a new fluoroquinolone antimicrobial, in the potential sites of pulmonary infection were assessed by fibreoptic bronchoscopy with bronchoalveolar lavage.

Fourteen patients received a course of temafloxacin, 600 mg twice daily, for three days prior to sampling. The mean serum concentration was 9.6 (SEM 1.2) mg·l^{-1}, compared with 14.9(SEM 1.8) mg·kg^{-1} for bronchial mucosa, 26.5 (SEM 3.6) mg·l^{-1} for epithelial lining fluid and 83.0 (SEM 11.5) mg·l^{-1} for alveolar macrophage. In the ten patients who completed the protocol, site concentrations correlated well with serum concentrations.

Temafloxacin was concentrated in each of the potential sites of infection examined and is, therefore, a promising new agent for the treatment of respiratory tract infection.

Eur Respir J, 1992, 5, 471–476

Dept of * Thoracic Medicine and
** Microbiology
Dudley Road Hospital
Birmingham, UK

Correspondence: D. Honeybourne
Dept of Thoracic Medicine
Dudley Road Hospital
Birmingham, UK.

Keywords: Fluoroquinolones
Pulmonary infection.

Received: July 5 1991
Accepted after revision December 3 1991.

Abbott Laboratories provided financial support for this study.

A recent approach to the assessment of antimicrobials has been to investigate the pharmacokinetics of new agents in potential sites of infection. This has been found to be necessary because of the great discrepancy which may exist between serum concentrations and those at other sites [1–4]. For some sites there is a clear relationship between local concentrations and clinical efficacy. Antimicrobials which concentrate in the urine are known to be effective even when serum concentrations would predict a clinical failure [5], this has also been noted for renal tissue [6, 7]. For the respiratory tract the situation is more complicated but evidence is accumulating that site concentrations are better predictors than serum concentrations. For example, some of the newer macrolide agents display *in vivo* activity which relates more closely to site concentrations than to their very low serum concentrations [8, 9].

The tissues of the respiratory tract previously studied include whole lung tissue, sputum and pleural fluid [10–12] but, unfortunately, these sites may be unrepresentative. Whole lung tissue consists of a number of different tissue compartments and, therefore, levels represent an average concentration. Sputum concentrations may be unreliable because of difficulties with sampling, contamination with blood and saliva, and sputum pooling over a number of hours thus producing antimicrobial instability and making conclusions about pharmacokinetics difficult [13–15]. Finally, pleural fluid is only likely to be a useful predictor of efficacy in empyema.

Antibiotic concentrations measured in bronchial mucosa (BM) are not subject to the same difficulties with sampling as sputum and may afford a more reliable prediction of clinical efficacy [16, 17]

By means of bronchoalveolar lavage, we can now investigate two further sites, namely the alveolar macrophage (AM) and epithelial lining fluid (ELF), which represent pure intracellular and pure extracellular sites, respectively. Important methodological factors have made the interpretation of antimicrobial concentrations in the AM and ELF difficult [18, 19], but with improved techniques this should become more meaningful [20–22].

Temafloxacin hydrochloride is a new fluoroquinolone with antibacterial activity approximating that of ciprofloxacin but with greater activity against *Streptococcus pneumoniae* [23, 24]. It has recently been shown to be effective in acute purulent exacerbations of chronic bronchitis, except where *Pseudomonas aeruginosa* was isolated [25]. In this study, we have investigated the concentrations of temafloxacin achieved in serum, BM, AM and ELF in patients taking a dose of 600 mg *b.d.*

Patients and methods

Fourteen patients (4 female) undergoing fibreoptic bronchoscopy for diagnostic purposes were included in the study. The final diagnoses were lung cancer in five, haemoptysis alone in four, and focal

radiological abnormality alone in four. Their mean age was 60 yrs (range 33–87 yrs) and weight 69.4 kg (range 52–83kg). Temafloxacin was administered in a dose of 600 mg *b.d.* for three days, with a final dose on the morning of bronchoscopy, making a total of seven doses. Exclusion criteria for the study were: 1) clinical or radiological evidence of cardiac failure; 2) disturbance of hepatic function (serum transaminase activity more than twice the upper limit of normal, bilirubin above the normal range); 3) renal impairment (blood urea greater than 9 $mmol \cdot l^{-1}$ or creatinine greater than 140 $\mu mol \cdot l^{-1}$); 4) a haemoglobin determination of less than 10 $g \cdot dl^{-1}$; 5) a history of concomitant drug therapy, including any other investigational drug, antibiotics, antacids, theophylline and drugs affecting the central nervous system other than sedatives 6) evidence of active lung infection; and 7) women with child-bearing potential. Subjects with active lung infection were excluded to ensure a homogeneous group.

All patients provided fully informed written consent and the study was approved by the Hospital Ethical Committee.

A blood biochemical profile (including urea, creatinine, calcium, bilirubin, aspartate transaminase, alkaline phosphatase and electrolyte determinations) and an automated Coulter count were performed before and after drug administration. Patients had a full physical examination before and after the study.

Sample collection

Fibreoptic bronchoscopy was performed using a premedication of 160 mg of nebulized 4% lignocaine and 0.6 mg of intramuscular atropine, and sedation with 3–7 mg of intravenous midazolam. After careful examination of the airways, "microlavage" (see below) was performed in a distal lung subsegment followed by a standard 200 ml bronchoalveolar lavage in a different lung segment (right middle lobe or lingula). Gentle aspiration was performed following each of the four 50 ml aliquots. The recovered fluid from the first 50 ml was discarded to avoid contamination with proximal airway cells [26]. Bronchial biopsies were then taken from macroscopically normal subcarinal mucosa (absence of erythema, induration or nodularity) in all patients. Lastly, a serum sample was taken for temafloxacin concentration, total protein and urea concentration, and for a red cell count (RCC).

"*Microlavage*"

This technique has been described previously [21, 22]. Briefly, a standard bronchial brush tube, 1.7 mm in external diameter, is inserted under direct vision into a distal subsegment and advanced until wedged. An assistant then injects 20 ml of normal saline rapidly, and immediate gentle aspiration is performed. The differential cell count obtained by microlavage agrees well with those of conventional bronchoalveolar lavage (BAL). A median of 3.4 ml of lavage fluid was recovered.

Calculation of epithelial lining fluid volume

A urea dilution method was used as described by RENNARD *et al.* [27]. This method was applied to the microlavage specimen, where the lavage dwell time was short enough for the urea to represent accurately that contained in the epithelial lining fluid (ELF) [28]. The concentration of total protein in the ELF was then determined as follows:

Microlavage:

$$\text{ELF volume} = \frac{[\text{urea}]\ \text{lavage} \times \text{volume lavage}}{[\text{urea}]\ \text{ELF}^*}$$

$$[\text{protein}]\ \text{ELF} = \frac{\text{volume lavage} \times [\text{protein}]\ \text{lavage}}{\text{ELF volume}}$$

* (= [urea] blood)

Therefore:

$$[\text{protein}]\ \text{ELF} = \frac{[\text{urea}]\ \text{ELF} \times [\text{protein}]\ \text{lavage}}{[\text{urea}]\ \text{lavage}}$$

The ELF protein concentration was then applied to the standard BAL to calculate the concentration of antibiotics in ELF recovered, since BAL protein varies far less with lavage dwell time [27]:

$$[\text{antibiotic}]\ \text{ELF} = \frac{[\text{protein}]\ \text{ELF} \times [\text{antibiotic}]\ \text{lavage}}{[\text{protein}]\ \text{lavage}}$$

The median volume of ELF recovered by microlavage was 0.78% of the lavage aspirate, whereas for conventional BAL the value was 1.5%.

Sample processing

The microlavage and standard BAL specimens were centrifuged immediately in the bronchoscopy suite, at 400 × g for 5 min, after a small aliquot had been removed for the total and differential cell counts. Total cell count preparations were again made immediately, and to avoid cell loss due to adherence to surfaces, siliconized glassware and teflon containers were used at all times. The BAL supernatant was freeze dried and then resuspended in distilled water to yield a tenfold concentration. Bronchial biopsies were collected into a humidity chamber and any blood-stained samples were discarded. Following addition of a known volume of cold phosphate buffer (pH 7.0), both the cell component and biopsies were ultrasonicated on ice for 2 min at 50% duty cycle (Ultrasonic Heat Systems W225).

The total white and red cell counts were performed using an Improved Neubauer haemocytometer. Samples were discarded if the red cell count was

greater than 4 × $10^5 \cdot ml^{-1}$. The differential count was performed on cellular monolayers prepared in triplicate by cytocentrifugation of 10^5 cells (100 µl of 10^6 white cells·ml^{-1}) at 450 rpm for 15 min (Shandon cytospin II). Slides were air-dried for 30 min prior to staining with May-Grünwald-Giemsa stain.

All samples were assayed for temafloxacin concentration using a microbiological plate assay in which the indicator strain (*E. coli* 4004 Bayer) was inoculated on to prepoured plates of Iso-Sensitest agar (Oxoid, Basingstoke, UK). Plates were incubated at 30°C for 18 h. Standards were prepared in phosphate buffer (pH 7.0) for cell and biopsy samples, in 9% saline for the concentrated supernatant, and in 100% human serum for serum samples. The between assay coefficient of variation was 7.9% and the lower limit of sensitivity 0.12 mg·l^{-1}. In control experiments homogenized AM and bronchial biopsies had no inhibitory effect on the indicator organism. The BAL supernatant and microlavage sample were also assayed for total protein and urea concentrations using modified Sigma diagnostic kits (Sigma Chemicals, Poole, UK). The urea assay measured blood urea nitrogen (Kit No. UV-66) and the sample to reagent ratio was adjusted to 1:1 instead of the 1:200 ratio recommended for serum samples. The assay was linear for the range 0.005–0.09 mmol·l^{-1}. Standards were prepared from a single quality control reference standard and diluted in normal saline. The between assay coefficient of variation was 7.8%.

Total protein was measured using Coomassie blue as an indication (Kit No. 610-A). The sample to reagent ratio was adjusted to 2:9. The standards were prepared as for the urea assay and the assay was linear over the range 0.005–0.10 g·l^{-1}. The between assay coefficient of variation was 7.1%.

Determination of AM volume

Velocity gradient centrifugation [29] was used to determine cell volume. This was found to agree with previous morphological studies [30] and with manual measurement of cell diameters employing microscopy of a hanging droplet. Using micropipettes to measure volume of displacement produced a 100% overestimate of cell volume and was not used. The mean cell volume was 2.48 (SEM 0.52) µl·10^{-6} cells.

Statistical method

Correlation between serum and site concentrations was analysed by calculating the specimen rank correlation coefficient.

Results

Figure 1 shows the concentrations of temafloxacin in serum, BM, ELF and AM plotted against time of last dose. Unfortunately, we were not able to sample bronchial mucosa and BAL in one patient due to technical difficulties and three patients did not complete a full course of therapy because of nausea, nausea and headache, and a skin rash resembling intertrigo. Two patients stopped 15 h before bronchoscopy, but still had detectable ELF, serum and AM levels. BM levels were detectable in only one of these patients. The third patient stopped 48 h before bronchoscopy, having had only 1,800 mg of temafloxacin. AM levels were still measurable at 6 mg·l^{-1}. In addition one patient experienced vomiting 1–2 h after the last four doses but still managed to take the full course. Consequently, temafloxacin levels were low and the results have been excluded from the calculation of means and medians.

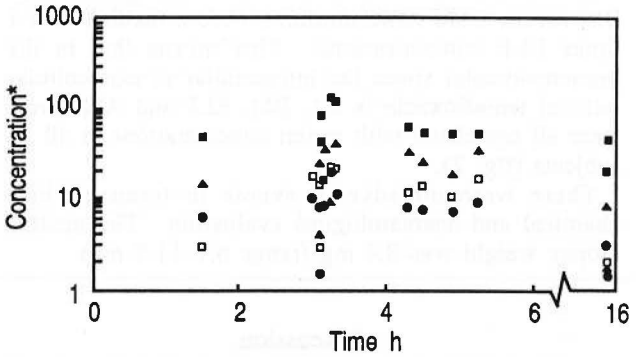

Fig. 1. – Bronchoalveolar distribution of temafloxacin. *Concentration: BM mg·kg^{-1}; serum, ELF, AM mg·l^{-1}. ● : Serum; □ : BM; ▲ : ELF; ■ : AM.

The concentrations and percentage penetrations are given in table 1 for the 10 patients who completed the protocol. The mean time since the final dose was 3.29 h (range 1.5–5.25 h) in these patients. There is good agreement between median and mean values, indicating that the data are not heavily skewed. BM levels were significantly higher than serum levels (mean difference 5.3 mg·l^{-1}; 95% confidence intervals 2.6, 8.0 mg·l^{-1}), ELF levels higher than BM levels (difference 14.2 mg·l^{-1}; 9.8, 18.6) and AM levels higher than ELF levels (difference 54.3 mg·l^{-1}; 31.1, 77.4).

Table 1. – Concentrations of temafloxacin in the lung

	Serum mg·l^{-1} n=10	BM mg·kg^{-1} n=10	ELF mg·l^{-1} n=9	AM mg·l^{-1} n=9
Concentrations				
Mean	9.6	14.9	26.5	83.0
SEM	1.2	1.8	3.6	11.5
Median	8.3	15.4	25.5	65.6
Range	6.2–20	2.9–22.9	9.3–41.2	49.3–138.7
Percentage penetrations				
Mean	-	156	306	881
SEM	-	14	39	78
Median	-	178	330	795
Range	-	47–193	46–430	600–1335

BM: bronchial mucosa; ELF: epithelial lining fluid; AM: alveolar macrophage.

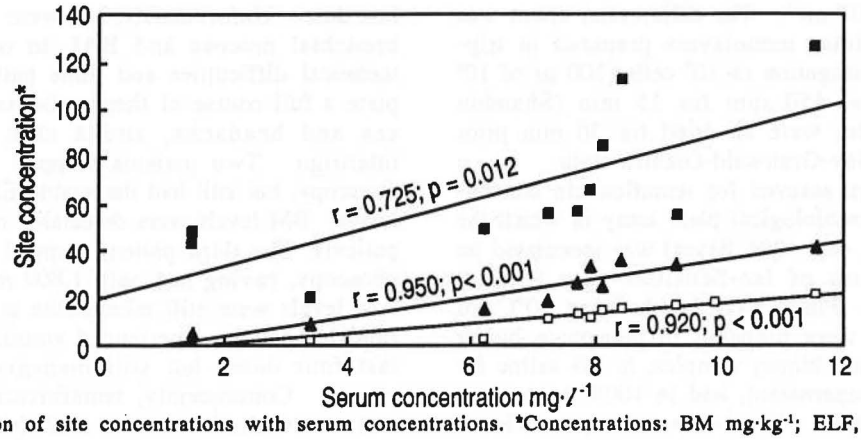

Fig. 2. – Correlation of site concentrations with serum concentrations. *Concentrations: BM mg·kg⁻¹; ELF, AM mg·l⁻¹. ■: BM; ▲: ELF; ■: AM.

ELF levels were a median of 1.9 times greater than BM levels. AM concentrations were a median of 3.1 times ELF concentrations. This means that in the bronchoalveolar space the intracellular to extracellular ratio of temafloxacin is 3:1. BM, ELF and AM levels were all correlated with serum concentrations in all 14 subjects (fig. 2).

There were no adverse events in terms of biochemical and haematological evaluation. The median biopsy weight was 8.4 mg (range 6.7–11.9 mg).

Discussion

The measurement of antimicrobial concentrations in the respiratory tract has been afflicted by two principal difficulties. Firstly, there have been well-documented problems with methodology [13–15, 18–22, 28], and secondly the necessity for measuring site concentrations has been questioned because it is argued that these can be predicted from serum levels [31]. Whilst it is possible to avoid blood and salivary contamination of sputum by aspirating directly through the bronchoscope [20], there remain the unavoidable problems of sputum pooling and instability of antimicrobials in sputum. For these reasons, we think measuring sputum concentrations is often meaningless, particularly where antimicrobial instability is a possibility. *In vitro* studies of AM antimicrobial concentrations have shown one potentially major source of error, which could lead to falsely low AM quinolone levels. When alveolar macrophages are allowed to take up antibiotics such as macrolides or quinolones *in vitro* and are then placed in "antibiotic free" media (in fact no more than a 1 in 100 dilution), there is a rapid efflux of antibiotic, such that for many drugs most of the intracellular drug is lost within 20 min [32, 33]. All quinolones tested by us and by others have been shown to efflux from cells very rapidly with conventional methodology [32, 34]. The *in vitro* efflux experiments are analogous to the situation in BAL. Here, normal saline dilutes the epithelial lining fluid antibiotic concentration by approximately 100 times. The aspirated BAL fluid thus contains macrophages in much diluted solution, and in the 4 min required to perform BAL, one would predict loss of most of the cell associated antibiotic. *In vitro* experiments suggest that a new equilibrium is set up which results in a cellular to extracellular antibiotic concentration ratio ranging from 4:1 to 10:1 [34]. However, in the present study we have measured macrophage concentrations which are 10,000 times those of the BAL supernatant, indicating that *in vivo* antibiotic efflux does not occur in the same way as that *in vitro*. AM to ELF ratios *in vivo* are consistent with those measured *in vitro* at 4–1. We have conducted further *in vitro* experiments to explain this phenomenon and will report these separately. For temafloxacin, prolonging the time from BAL to separation of the cell pellet by centrifugation causes only a minimal reduction of cell associated drug and we therefore assume that efflux is not a significant problem, provided that centrifugation is not delayed beyond 10 min. Our samples were separated immediately in the bronchoscopy suite.

The difficulty with the measurement of ELF antimicrobial concentrations is the determination of the ELF volume recovered in lavage fluid. Previously, workers have used a urea dilution method [27] to calculate this but an overestimation of over 100% results, as urea moves into lavage fluid during the prolonged dwell times required for conventional lavage [28]. The microlavage technique lavages a distal subsegment of the lung within 20 s, thus circumventing the problem of prolonged dwell time. The microlavage tube is only 1.7 mm in diameter and since respiratory epithelium lines airways of less than 2 mm diameter it is not surprising that this procedure gives similar differential cell count to conventional BAL. We can expect, therefore, that ELF recovered by microlavage is representative of that recovered by conventional BAL when the aspirate from the first 50 ml is discarded.

It has been suggested that for the lung, clinical efficacy can be best predicted by serum concentrations rather than site concentrations [31]. Indeed, a correlation of serum concentration and eradication of pathogens aspirated from endotracheal tubes has been noted [32]. However, the efficacy of the newer macrolide agents is against this hypothesis because they

have established clinical efficacy despite serum concentrations which are below minimal inhibitory concentration (MIC_{90}) values for many pathogens [8, 9]. The prediction of antimicrobial levels in sites within the lung from serum levels is not possible, except perhaps for bronchial mucosa [31]. For BM, interstitial fluid antimicrobial levels are likely to be approximately the same as serum, because the two compartments are only separated by the rather "leaky" non-fenestrated capillaries. Therefore, if the approximate intracellular:extracellular ratio of drug is known, and the ratio of extracellular water:total water is known, then the drug levels can be predicted. However, this is not the situation for either ELF levels, or AM levels, because they are separated from the blood by the alveolar epithelium, which has been shown to be a relatively impermeable membrane, approximating to an intact cell membrane [35, 36].

The relevance of assessing drug concentrations in healthy lung has rightly been questioned because it is argued that the concentrations in inflamed tissues may be different and that these may be more appropriate as predictors of clinical efficacy. However, in any infection there is healthy tissue bordering the inflamed area where antimicrobial levels may be important to prevent microbial spread. In addition, the barriers to antimicrobial movement may regain their integrity during the stages of resolution, so that poor penetration of antimicrobial into healthy tissue might lead to relapse. Measuring antimicrobial concentrations in inflamed sites of infection in the lung poses further methodological and ethical problems. There are no methods available to estimate epithelial lining fluid volume in inflamed lung and the cell differential count in the lung may be markedly different, with neutrophils predominating in bacterial infections. Fibreoptic bronchoscopy and bronchoalveolar lavage may be unethical in cases of pneumonia where the procedure is not clinically indicated.

The median bronchial mucosal concentration of temafloxacin found in the present study of 179% compared very well with our previous finding of 178% for this drug [37] and is similar to other quinolones [17] (except in the unconfirmed results with enoxacin [38]). Our findings that temafloxacin is concentrated in the ELF compared to serum and BM could not have been predicted solely from the knowledge that quinolones are concentrated inside cells because although the alveolar membrane is similar in its permeability characteristics to a cell membrane, the ELF is not likely to be the same as the intracellular environment. It is, therefore, essential to continue to measure ELF levels of drugs because this site may be more applicable to measure *in vitro* activity and, therefore, potentially, a good predictor of clinical response. It is of interest that the levels found in ELF were all well above the MICs of the pneumococci isolated by DAVIES *et al.* [25], except for the one resistant strain which was a treatment failure.

The exact levels within the AM could not be predicted, but it may be useful to have particularly high levels for effective treatment of intracellular infections. The levels achieved in AM should be compared with those required to kill intracellular pathogens *in vitro* [39, 40], because intracellular concentrations may not have a direct relationship to intracellular killing [41]. This is a consequence of compartmentalization of antimicrobials in sites separate from the pathogens. Temafloxacin is a promising agent for treatment of respiratory tract infections because of excellent tissue concentration shown by this study.

References

1. Chisholm GD, Waterworth PM, Calnan JS, Garrod LP. – Concentration of antibacterial agents in interstitial tissue fluid. *Br Med J*, 1973; 1: 596–573.
2. Halprin GM, McMahon SM. – Cephalexin concentrations in sputum during acute respiratory infections. *Antimicrob Agent Chemother*, 1973; 3: 703–707.
3. Norrby RA. – Review of the penetration of antibiotics into CSF and its clinical significance. *Scand J Infect Dis*, 1978; 14 (Suppl.): 296–309.
4. Rolinson GN. – Tissue penetration of antibiotics. *J Antimicrob Chemother*, 1984; 13: 593–602.
5. Stamey TA, Fair WR, Timothy MM, Millar MA, Mihwa G. – Serum *vs* urinary antimicrobial concentrations in cure of urinary tract infections. *New Engl J Med*, 1974; 291: 1159–1163.
6. Fabre J, Rudhart M, Blanchard P, Reganey C. – Persistence of sisomycin and gentamicin in renal cortex and medulla compared to other organs and serum in rats. *Kidney International*, 1976; 10: 444–449.
7. Glauser MP, Lyons JM, Braude AI. – Prevention of pyelonephritis due to *E. coli* in rats with gentamicin stored in kidney tissue. *J Infect Dis*, 1979; 139: 172–177.
8. Girard A, Girard D, Retsema J. – Correlation of the extravascular pharmacokinetics of azithromycin with *in vivo* efficacy in models of localized infection. *J Antimicrob Chemother*, 1990; 25 (Suppl. A): 61–71.
9. Davies BI, Maesen FPV, Gubbelmans S. – Azithromycin (CP-62, 993) in acute exacerbations of chronic bronchitis: and open clinical, microbiological and pharmacokinetic study. *J Antimicrob Chemother*, 1989; 23: 743–751.
10. Kiss IJ, Farago E, Pinter J. – Serum and lung tissue levels of cephradine in thoracic surgery. *Br J Clin Pharmacol*, 1976; 3: 891–895.
11. Kroenig U, Liebig S, Wundschock M. – Tombracin concentration in human lung tissue. *Infections*, 1978; 6: 231–235.
12. Cole DR, Pung J. – Penetration of cefazolin into pleural fluid. *Antimacrob Agents Chemother*, 1977; 1: 1033–1035.
13. Pennington JE. – Penetration of antibiotics into respiratory secretions. *Rev Infect Dis*, 1981; 3: 67–73.
14. Stockley RA, Dragicevic P, Burnett D, Hill SL. – Role of β-lactamases in the response of pulmonary infections to amoxycillin/clavulanate. *J Antimicrob Chemother*, 1989; 23 (Suppl. B): 73–81.
15. Bergogne-Berezin E. – Penetration of antibiotics into respiratory secretions. *J Antimicrob Chemother*, 1981; 8: 171–174.
16. Marlin GE, Burgess KR, Burgoyne J, Funnel GR, Guiness MDG. – Penetration of piperacillin into bronchial mucosa and sputum. *Thorax*, 1981; 36: 774–780.

17. Honeybourne D, Andrews JM, Ashby JP, Lodwick R, Wise R. – Evaluation of the penentration of ciprofloxacin and amoxycillin into the bronchial mucosa. *Thorax*, 1988; 43: 715–719.
18. Chastre J, Brun P, Fourtillan JB, Soler P, Basset G, Manuel C, Trouillet JL, Gubert C. – Pulmonary disposition of roxithromycin (RU 29865), a new macrolide antibiotic. *Antimicrob Agent Chemother*, 1987; 31: 1312–1316.
19. Panteix G, Harf R, de Montclos H, Verdier A, Gaspar A, Leclercq M. – Josamycin pulmonary penetration determined by bronchoalveolar lavage in man. *J Antimicrob Chemother*, 1988; 22: 917–921.
20. Baldwin DR, Wise R, Andrews JM, Ashby JP, Honeybourne D. – Azithromycin concentrations at the sites of pulmonary infection. *Eur Respir J*, 1990; 3: (in press).
21. Baldwin DR, Wise R, Andews JM, Honeybourne D. – "Microlavage" - a technique for the determination of epithelial lining fluid volume. *Thorax*, 1990; 45: 823–824.
22. Baldwin DR, Wise R, Andrews JM, Honeybourne D. – Measurement of epithelial lining fluid volume employing a new technique - "microlavage". *Eur Respir J*, 1990; 3 (10s): 105s–106s.
23. Nye K, Shi YG, Andrews JM, Ashby JP, Wise R. – The *in vitro* activity, pharmacokinetics and tissue penetration of temafloxacin. *J Antimicrob Chemother*, 1989; 24: 415–424.
24. Barry AL, Jones RN. – *In vitro* activities of temafloxacin, tosufloxacin (A-61827) and five other fluoroquinolone agents. *J Antimicrob Chemother*, 1989; 23: 527–535.
25. Davies BI, Maesen FPU, Gabbelmans HLL, Cremers HMHG. – Temafloxacine in acute purulent exacerbations of chronic bronchitis. *J Antimicrob Chemother* 1990; 26: 237–246.
26. Kelly CA, Kohe JC, Ward C, Hendrick DJ, Walters EH. – Anatomical distribution of bronchoalveolar lavage fluid as assessed by digital subtraction radiography. *Thorax*, 1987; 42: 626–629.
27. Rennard SI, Basset G, Lecossier D, O'Donnell KM, Pinkston P, Martin G, Crystal RG. – Estimation of volume of epithelial lining fluid recovered by lavage using urea as a marker of dilution. *J Appl Physiol*, 1986; 60(2): 532–538.
28. Marcy TW, Merrill WW, Rankin JA, Reynolds HY. – Limitations of using urea to quantify epithelial lining fluid recovered by bronchoalveolar lavage. *Am Rev Respir Dis*, 1987; 135: 1276–1280.
29. Johnson JD, Hand WL, Francis JB, King-Thompson N, Corwin RW. – Antibiotic uptake by alveolar macrophages. *J Lab Clin Med*, 1980; 95: 429–439.
30. Crypo JD, Barry BE, Behr R, Bachofen M, Weibel ER. – Cell numbers and cell characteristics of the normal human lung. *Am Rev Respir Dis*, 1982; 126: 332–337.
31. Schentag JJ. – Clinical significance of antibiotic tissue penetrations. *Clin Pharmacokinetics*, 1989; 16 (Suppl. 1): 25–31.
32. Johnson JD, Hand WL, Francis JB, King-Thompson N, Corwin RW. – Antibiotic uptake by alveolar macrophages. *J Lab Clin Med*, 1980; 95: 429–439.
33. Hand WL, King-Thompson N. – Membrane transport of clindamycin in alveolar macrophages. *Antimicrob Agents Chemother*, 1982; 21: 241–247.
34. Easmon CSF, Crane JP. – Uptake of ciprofloxacin by human neutrophils. *J Antimicrob Chemother*, 1985; 16: 67–73.
35. Taylor AE, Guyton AC, Bishop VS. – Permeability of the alveolar membrane to solutes. *Circ Res*, 1965; 16: 353–361.
36. Effros RM, Mason GR, Silverman P, Reid E, Hukkanen J. – Movement of ions and small solutes across endothelium and epithelium of perfused rabbit lungs. *J Appl Physiol*, 1986; 60: 100–107.
37. Baldwin DR, Wilkinson L, Andrews JM, Ashby JP, Wise R, Honeybourne D. – Concentrations of temafloxacin in serum and bronchial mucosa. *Eur J Clin Microbiol Infect Dis*, 1990; 9: 432–434.
38. Marlin GE, Braude PD, Wheton AJ, Somoggi AA. – Penetration of enoxacin into human bronchial mucosa. *Am Rev Respir Dis*, 1986; 134: 1209–1212.
39. Jacobs RF, Wilson CB. – Intracellular penetration and antimicrobial activity of antibiotics. *J Antimicrob Chemother*, 1983; 12 (Suppl. C): 13–20.
40. Steinberg TH, Hand WL. – Effect of phagocyte membrane stimulation of antibiotic uptake and intracellular bactericidal activity. *Antimicrob Agents Chemother*, 1987; 31: 660–662.
41. Hand WL, King-Thompson NL. – Contrasts between phagocytic antibiotic uptake and subsequent intracellular bactericidal activity. *Antimicrob Agents Chemother*, 1986; 29: 135–140.