BIOPHARMACEUTICS & DRUG DISPOSITION, VOL. 16, 393–401 (1995)

EVALUATION OF THE EFFECTS OF AMBROXOL ON THE OFLOXACIN CONCENTRATIONS IN BRONCHIAL TISSUES IN COPD PATIENTS WITH INFECTIOUS EXACERBATION

FABRICE PAGANIN*, OLIVER BOUVET†, PASCAL CHANEZ*, DAVID FABRE†, MARC GALTIER†, PHILIPPE GODARD*, FRANÇOIS B. MICHEL* AND FRANÇOISE BRESSOLLE‡§

*Département des Maladies Respiratoires, Hopital Arnaud de Villeneuve, Université de Montpellier I, France
†Laboratoire de Pharmacocinétique, Pharmacie Carémeau, CHU Nîmes, France
‡Département de Pharmacocinétique, Faculté de Pharmacie, Université de Montpellier I, France

ABSTRACT

Infectious excerbations of COPD are generally due to *Streptococcus pneumoniae*, *Haemophilus* species, and other Gram-negative bacteria. Ofloxacin has potent activity against Gram-negative species but is less effective against Gram-positive species including *Streptococcus pneumoniae*. It has also been shown that the administration of ambroxol increases the concentration of some antibiotics in pulmonary tissues. The aim of the study was to determine whether ambroxol increases the bronchial tissue concentrations of ofloxacin to a level exceeding the MIC_{90} of the bacterial species less susceptible to ofloxacin. 24 patients with COPD were randomized in two groups. Drug regimens of ofloxacin alone (200 mg twice daily) or ofloxacin (200 mg twice daily) + ambroxol (30 mg thrice daily) were administered over 10 d. A fibroscopy was performed on day 10 with bronchial biopsies and broncho-alveolar lavage. At steady state, concentrations of drug in plasma and bronchial samples were assayed by HPLC with fluorometric detection. There was no significant difference in the bronchial levels of ofloxacin between the two groups; however, in alveolar cells, ofloxacin concentration was three times higher in the group with ambroxol. Ambroxol does not increase ofloxacin concentrations in bronchial tissue because high concentrations are already present in the lung.

KEY WORDS: ofloxacin; ambroxol; bronchial biopsies; tissue penetration; COPD

INTRODUCTION

Infectious exacerbation in chronic obstructive pulmonary disease (COPD) patients is frequent and is associated with an increase in morbidity. In this context many bacterial species have been implicated as etiologic pathogen

§Corresponding author and reprint requests: Pr. Françoise Bressolle, Département de Pharmacocinétique, Faculté de Pharmacie, Université de Montpellier I, France.

CCC 0142–2782/95/050393–09

Received 18 January 1993
Accepted 5 January 1995

agents, i.e. *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Moraxella catarrhalis*, and more rarely other Gram-negative species as *Pseudomonas*, *Klebsiella*, *Escherichia coli*, and other Enterobacteriaceae. Ofloxacin is a fluoroquinolone characterized by a high level of *in vitro* activity against a wide range of pathogens such as Gram-negative bacilli and staphylococcus but less against *Streptococcus* species. The minimal inhibitory concentrations (MICs) of ofloxacin for 90% of these bacteria range from 0·015 to 4 mg L^{-1}.[1] Ofloxacin has been therefore used successfully in clinical trials for infectious exacerbations in COPD patients.[2] Mucolytic agents are currently used in patients with COPD to facilitate lung mucociliary clearance and therefore to help in the removal of bronchial secretions.[3] Moreover, previous studies have shown that mucolytic agents increase the penetration of several antibiotics (ampicillin, amoxicillin, erythromycin) in the bronchi and the lungs.[4] Ambroxol (*trans*-[(amino-2-dibromo-3, 5 benzyl) amino]-4-cyclohexanol chlorhydrate, Boehringer Ingelheim Reims, France), is a molecule with two major properties, namely the ability to increase mucociliary transport and to increase the synthesis of surfactant. It has been shown previously that ambroxol increases the concentration of several antibiotics in pulmonary tissues.[5]

The aim of this study was to determine whether ambroxol increases the bronchial tissue concentrations of ofloxacin to a level exceeding the MIC_{90} of the bacterial species less susceptible to ofloxacin.

MATERIALS AND METHODS

Patients

The study was carried out in 24 patients (19 males and five females) aged from 48 to 73 years (mean 61·5 ± 7·4) undergoing diagnostic fibre optic bronchoscopy. Mean body weight was 70·1 ± 9·2 kg. All of them had COPD according to the American Thoracic Society (ATS) criteria.[6] The diagnosis of infection was based on physical examination and symptoms of productive cough, dyspnea, and purulent expectoration. The status of the patients was ascertained by standard laboratory tests. These tests were performed on day 1 before treatment and the last day of the experiment. The following pulmonary function tests: forced expiratory volume in one second (FEV_1), and FEV_1 divided by vital capacity (FEV_1/VC%) were performed before and after the experiment using a Pneumoscreen (E. Jaeger Lab, Würtzburg, Germany). Ofloxacin (200 mg/12 h) and ambroxol (30 mg/8 h) were administered orally for 10 d to obtain steady-state concentrations before the pharmacokinetic study. The patients were informed of the study design and were enrolled after having given written informed consent. The study protocol was in accordance with the legal requirements of the declaration of Helsinki and was approved by the local hospital ethics committee. The order of patients was randomly assigned.

Sputum examination

Sputum samples were obtained before administration of drug to confirm the diagnosis of infectious exacerbation. More than 25 leucocytes and less than five squamous epithelial cells was considered as a bronchial specimen. Bacteria that grew at a 10^{-5} dilution were considered significant. These were identified and tested for sensitivity using the comparative disc diffusion technique for ofloxacin.

Blood and tissue samples

Blood samples, collected on day 10 of treatment in tubes coated with ethylenediaminetracetic acid (EDTA), were withdrawn from a venous radial catheter immediately predose and at 60 min, 180 min (the time of the tissue sample), and 6 h post-dose. Plasma was separated from blood by centrifugation (2000*g* for 5 min) and was stored at −80 °C until analysis. On day 10 of treatment, bronchial biopsies were carried out by fibre optic bronchoscopy (BF20 Olympus) using alligator forceps in a subsegmental bronchus of the left inferior lobe. Biopsies were washed for 30 s in 0·9% sodium chloride solution in order to limit blood contamination, then dried on gauze before storage at −80 °C. Broncho-alveolar lavage (BAL) was performed in the middle lobe and a total of 150 mL of sterile warm saline solution was instilled in three aliquots of 50 mL. The fluid was recovered by gentle syringe suction. The first aliquot was discarded and the two others were pooled, as suggested by several investigators.[7] The lavage fluid was centrifuged at 2500*g* for 15 min. Alveolar cells and supernatant were stored subsequently at −80 °C.

Assay method

The concentrations of ofloxacin in plasma, biopsy specimens, and the two components of BAL were determined by a high-performance liquid chromatography (HPLC) procedure with fluorescence detection.[8] The limit of quantitation of the method was 0·0125 $\mu g\,mL^{-1}$ with a precision of 20%. Quality control samples were included in each analytical sequence to verify the stability of the study samples during storage and the accuracy and precision of ofloxacin analysis. In all cases, the relative error was less than 5%.

Statistical analysis

Throughout the presentation of the results, individual parameters and means ± SD are given. Observed plasma concentrations were plotted against corresponding tissue concentrations. Linear regression analysis was performed using the unweighted least-squares analysis of the data. The significance of the regression was confirmed by the *F* test. A non-parametric test (the Kruskall–

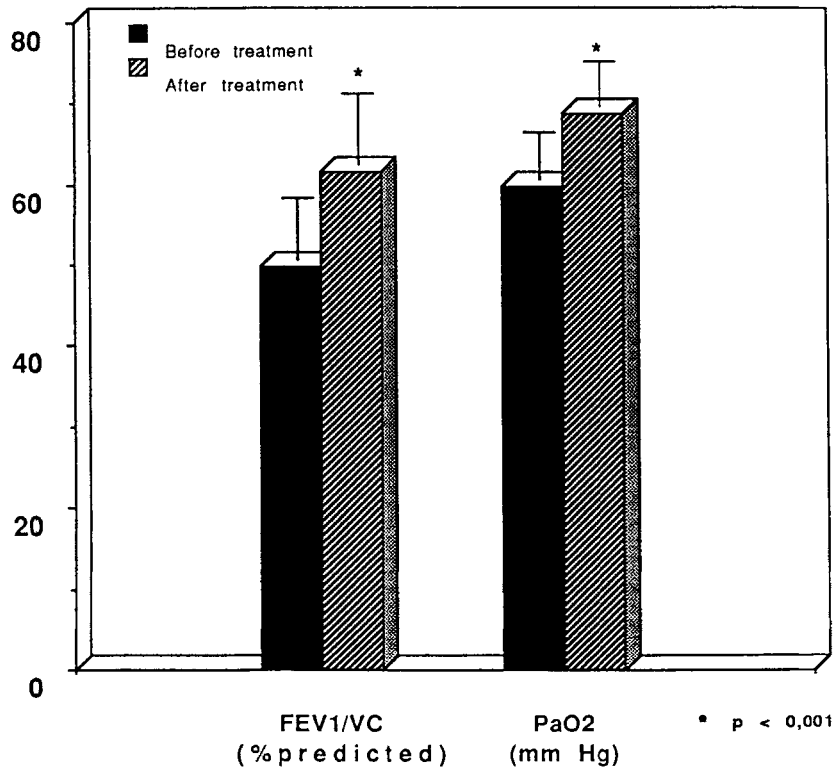

Figure 1. Evolution of the lung function parameters in the patients after 10 d of treatment

Wallis test) was used to compare the values obtained in the two groups of patients. The Wilcoxon test was used to compare the pulmonary function tests and blood gases before and after treatment. A p value of less than 0·05 was considered significant.

RESULTS

Among the 24 patients, 20 were evaluable. The two groups of patients did not differ significantly with regard to demographic data and all the patients improved clinically. The increase of FEV_1/VC and PaO_2 after treatment was statistically significant ($p < 0{\cdot}001$) (Figure 1). The individual strains isolated and the corresponding *in vitro* sensitivity are reported in table 1. Individual results are reported in Table 2 and summarized in Figure 2. The mean peak plasma concentration value was reached 1 h after drug administration (Table 2). The diffusion of ofloxacin is notable, 3 h post-dose, on day 10, levels of the drug in bronchial mucosa and alveolar cells ranged from 0·0125 to 5·21 $\mu g\, g^{-1}$

Table 1. Bacteriological data in the two groups of patients. MIC values are expressed in milligrams per litre

	Pathogen species	MIC_{90}: ofloxacin Measured	MIC_{90}: ofloxacin Range (mean *in vitro*)	Comments
Group 1				
1	*Haemophilus influenzae*	0·5	0·015–0·06	*In vitro* ofloxacin resistance
2	*Staphylococcus aureus*	2	0·12–1	*In vitro* ofloxacin resistance
3	Moraxella cat	0·05	0·03–0·12	
4	*Haemophilus influenzae*	1	0·015–0·06	*In vitro* ofloxacin resistance
5	*Streptococcus pneumoniae*	2	1·2	*In vitro* ofloxacin resistance
6	*Streptococcus alpha*	ND	0·25–1	No pathogen
7	*Pseudomonas aer.*	0·4	0·5–4	
8	*Streptococcus salivarius*	ND	0·25–1	No pathogen
9	*Streptococcus pneumoniae*	1	1–2	Low activity of ofloxacin
10	*Haemophilus influenzae*	0·5	0·015–0·06	*In vitro* ofloxacin resistance for *Haemophilus*
	Klebsiella pneumoniae	1	0·25–1	
11	*Pseudomonas aer.*	0·3	0·5–4	
12	*Streptococcus pneumoniae*	NA	1–2	Positive DE, no growth in culture
Group 2				
1	*Streptococcus sanguis*	2	1–4	
2	*Streptococcus pneumoniae*	1	1–2	Low activity of ofloxacin
3	*Haemophilus influenzae*	1	0·015–0·06	*In vitro* ofloxacin resistance
4	*Haemophilus influenzae*	0·03	0·015–0·06	
5	*Pseudomonas aer.*	0·6	0·5–4	
6	*Streptococcus alpha*	ND	0·25–1	No pathogen
7	*Haemophilus influenzae*	0·25	0·015–0·06	Low activity of ofloxacin
	Staphylococcus aureus	0·25	0·12–1	
8	*Haemophilus aphrophilus*	0·5	0·015–0·06	*In vitro* ofloxacin resistance

DE, direct examination; NA, not available; ND, not done.

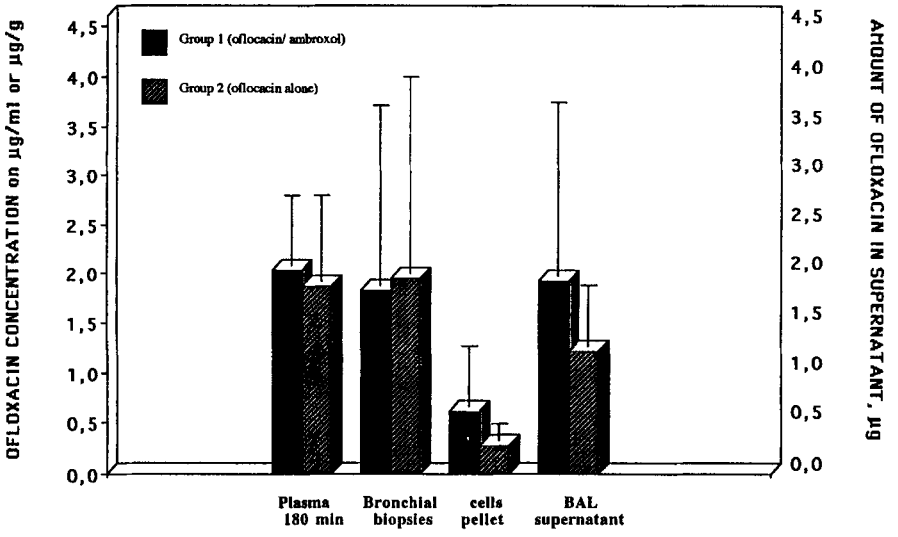

Figure 2. Ofloxacin concentrations in different assays: comparative data between groups 1 and 2

and from 0·097 to 1·61 $\mu g\,g^{-1}$, respectively, in group 1 and from 0·0125 to 5·7 $\mu g\,g^{-1}$ and from 0·149 to 0·55 $\mu g\,g^{-1}$, respectively in group 2, resulting in mean tissue/plasma ratios of $1 \pm 1{\cdot}16$ and $0{\cdot}37 \pm 0{\cdot}39$, respectively, in group 1, $1{\cdot}13 \pm 1{\cdot}09$ and $0{\cdot}29 \pm 0{\cdot}29$, respectively, in group 2. The non-parametric test showed no significant difference between the two groups. However, the mean value of the alveolar cells was about three times higher in group 1 than in group 2, $0{\cdot}62 \pm 0{\cdot}5\,\mu g\,g^{-1}$ compared with $0{\cdot}27 \pm 0{\cdot}16\,\mu g\,g^{-1}$. Ofloxacin concentrations in tissues were not correlated with the corresponding plasma concentrations.

DISCUSSION

The efficacy of ofloxacin was demonstrated in that after 10 d of treatment, all the patients showed clinical improvement. The pathogens isolated in the present study were in accordance with those generally found in the literature on COPD patients with infectious exacerbation.[9] However, the clinical efficacy observed might be questionable considering the bacteriological data obtained, because we found some strains *in vitro* resistant to ofloxacin. Taking into account the levels of ofloxacin that involve the total amount of the drug (free and bound), and the low serum protein binding of ofloxacin (10%), improvement might be explained by the concentrations reached in the bronchial tissue and in BAL, which far exceeded the MIC for 90% of the bacterial isolates involved in our patients. These data confirmed previous

Table 2. Concentrations of ofloxacin in the different assays

	Plasma[a] 0	Plasma[a] 60 min	Plasma[a] 180 min	Plasma[a] 6 h	Biopsy/ plasma	Alveolar cells/ plasma	Supernatant[b] (μg)
Group 1							
OFLOXACIN +	0·139	2·75	2·27	2·1	0·125	0·71	2·94
AMBROXOL $n=12$	< LOQ	3·68	1·87	NA	2·529	0·187	1·54
	1·29	3·15	1·99	1·88	0·216	0·097	0·74
	1·38	4·18	3·04	2·9	0·312	0·089	1·26
	0·34	1·51	1·21	1·11	NA	1·09	1·35
	1·04	3·61	1·9	1·62	0·173	0·778	6·76
	1·02	2·88	1·78	1·62	0·282	0·134	1·73
	1·06	3·88	3·28	NA	NA	0·06	1·15
	0·118	1·54	1·33	0·91	0·28	0·939	0·115
	0·554	1·89	1·51	1·48	3·461	0·144	2·6
	1·58	3·57	2·95	2·88	1·34	0·033	0·4
	< LOQ	1·67	1·23	0·94	1·26	0·119	2·58
Mean ± SD	0·71 ± 0·58	2·86 ± 0·98	2·03 ± 0·72	1·74 ± 0·72	1 ± 1·16	0·37 ± 0·39	1·93 ± 1·75
Group 2							
OFLOXACIN ALONE	2·10	6·25	1·87	5·2	NA	NA	0·665
$n=8$	0·995	3·09	2·03	1·79	1·19	0·273	1·1
	< LOQ	2·1	1·25	0·39	0·328	0·146	2·03
	< LOQ	0·99	0·925	0·913	0·421	0·188	0·79
	0·85	1·8	1·74	1·31	3·28	0·908	1·23
	0·453	2·31	1·91	1·39	0·654	0·155	0·88
	0·845	2·17	1·57	1·52	NA	0·326	2·29
	2	4·64	3·66	3·03	0·887	0·04	0·7
Mean ± SD	0·91 ± 0·80	2·92 ± 1·72	1·87 ± 0·88	1·94 ± 1·52	1·13 ± 1·09	0·29 ± 0·29	1·21 ± 0·62

[a]Plasma concentration in ($\mu g\,mL^{-1}$).
[b]Amount of ofloxacin in supernatant.
NA; not available; LOQ, limit of quantitation.

studies of high quinolone penetration in bronchi and lung cells.[10] The assessment of antibiotic tissue concentrations using bronchial biopsies and BAL is a new procedure. Some studies have been able to measure antibiotic levels in the sputum,[11] but there are difficulties in standardizing the samples and in avoiding contamination with saliva and squamous cells. BAL has been shown to reflect the alveolar cell pattern. In patients with infectious bronchitis, the absolute number of cells and the percentage of polynuclear neutrophil cells recovered by BAL increased significantly. The supernatant of BAL is made up of the saline infusion, epithelial lining fluid, and a degree of bronchial secretions. Bronchial biopsies are useful to obtain tissue for drug measurement. The quality of our sampling allowed reproducible antibiotic tissue concentration analysis. This method reflects the exact antibiotic concentration occuring in the bronchial tissue. In spite of the small number of patients, the mean value of ofloxacin concentration in alveolar cells is three times higher when ofloxacin is associated with ambroxol than alone ($0{\cdot}62\,\mu g\,mL^{-1}$ compared with $0{\cdot}27\,\mu g\,mL^{-1}$). Nevertheless this difference was not statistically significant. However, the alveolar cells are affected by pathogenic bacteria in the case of pneumonitis and in this case our results suggest that the association ofloxacin/ambroxol could be effective. However, this consideration should be taken cautiously and the clinical relevance is questionable. A possible explanation for the BAL data is a rapid efflux of quinolones from alveolar cells to the extracellular compartment. The new quinolones, given orally are valuable and relatively safe for the treatment of patients with respiratory infections. The role of ambroxol as a pharmacokinetic vector to increase the pulmonary tissue concentrations of ofloxacin is rather weak. This is due to the pharmacokinetic properties of ofloxacin and other 4-quinolones, which reach high tissue concentrations alone, unlike other antibiotics for which tissue concentrations are improved by ambroxol.

ACKNOWLEDGEMENTS

The authors would like to thank Dr Hélène Darbas (Département de Bactériologie, Hopital A de Villeneuve, Montpellier) and Mrs Allison Campbell for helpful discussion and reviewing the manuscript.

REFERENCES

1. R. N. Grüneberg, D. Felmingham, M. D. O'Hara *et al.*, The comparative *in vitro* activity of ofloxacin *J. Antimicrob. Chemother.*, **22**, suppl. C, 9–19 (1988).
2. F. P. V. Maesen and B. I. Davies, Quinolones antimicrobial agents in acute exacerbations of chronic bronchitis. *Eur. J. Resp. Dis.*, **69**, suppl. 146, 585–590 (1986).
3. E. J. Morgan and T. L. Petty, Summary of the national mucolytic study. *Chest*, **97**, 24s–27s (1990).

4. J. C. Wiemeyer, Influence of ambroxol on the pulmonary level of antibiotics. *Drug Res.*, **31**, 974–976 (1981).
5. J. Spatola, J. J. Poderosso, J. C. Wiemeyer *et al.*, Influence of ambroxol on lung tissue penetration of amoxicillin. *Drug Res.*, **37**, 965–966 (1987).
6. American Thoracic Society, Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. *Am. Rev. Respir. Dis.*, **136**, 225–244 (1987).
7. BAL Cooperative Group Steering Committee, Bronchoalveolar lavage constituents in healthy individuals, idiopathic pulmonary fibrosis, and selected comparison groups. *Am. Rev. Respir. Dis.*, **141**, S169–S170 (1990).
8. G. Montay and J. P. Tassel, Improved high performance liquid chromatographic determination of pefloxacin in human plasma and tissue. *J. Chromatogr.*, **339**, 214–218 (1985).
9. R. A. Walstad, Interventions in obstructive lung disease: the effects of antibiotics. *Eur. Respir. Rev.*, **1**, 387–391 (1992).
10. W. J. A. Wijnands, T. B. Vree, A. M. Baars *et al.*, The penetration of ofloxacin into lung tissue. *J. Antimicrob. Chemother.*, **22**, suppl. C, 85–89 (1988).
11. J. Symonds, A. Javaid, M. Bone *et al.*, The penetration of ofloxacin into bronchial secretions. *J. Antimicrob. Chemother.*, **22**, suppl. C, 91–95 (1988).