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The concentrations of clarithromycin and its 14-hydroxy metabolite in sputum of patients with bronchiectasis following single dose oral administration

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Clarithromycin and its metabolite, 14-hydroxy-clarithromycin are active against a wide range of respiratory pathogens. Antibiotics generally penetrate poorly into respiratory secretions which may therefore continue to harbour bacteria following bronchial infection. We have studied sputum and serum concentrations of clarithromycin and 14-hydroxy-clarithromycin in eight patients with idiopathic bronchiectasis without infective exacerbations (five male, three female; mean age 53·3 years). Oral single dose administration of 250 or 500 mg clarithromycin, separated by at least 6 days, was given to each patient. Serum and sputum samples were collected (the latter by physiotherapy at 0, 1, 2, 4, 8, 24 and 0, 4, 8 and 24 h respectively after administration of each dose. Serum sol phase was obtained by high speed centrifugation and concentrations of clarithromycin and 14-hydroxy-clarithromycin were determined by high performance liquid chromatography. Serum C_{max} for clarithromycin and 14-hydroxy-clarithromycin were 1·20 mg/L (3 h) and 0·37 mg/L (3·1 h) for clarithromycin (250 mg)) and were 2·78 mg/L (2·5 h) and 0·68 mg/L (2·6 h) for clarithromycin (500 mg) respectively. Sputum C_{max} for clarithromycin and 14-hydroxy-clarithromycin were 0·52 mg/L (5 h) and 0·30 mg/L (6·5 h) for clarithromycin (250 mg) and were 1·59 mg/L (5 h) and 0·47 mg/L (5·5 h) for clarithromycin (500 mg) respectively. The sputum/serum percentage ratios at C_{max} (sputum) for clarithromycin and 14-hydroxy-clarithromycin were 74·3% and 113·9% (250 mg) and 94·7% and 99·9% (500 mg) respectively. We conclude that oral administration of clarithromycin to patients with bronchiectasis results in rapid penetration into respiratory mucus with persistent drug concentrations that exceed its MIC for many respiratory pathogens.

Introduction

Clarithromycin is a new macrolide antibiotic which is a 6-methoxy derivative of erythromycin. Clarithromycin is more resistant to gastric acid and has a better pharmacokinetic profile than erythromycin. A metabolite of clarithromycin, 14-hydroxy-clarithromycin, also has antibiotic activity against *Haemophilus influenzae*. Clarithromycin and 14-hydroxy-clarithromycin have an additive effect which reduces the MIC for this bacterium (Hardy *et al.*, 1990). Clarythromycin has been shown to be effective in patients with infective exacerbations of chronic obstructive lung disease (Guay & Craft, 1992).

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Recent studies (Farley *et al.*, 1986; Baltimore, Christie & Walker-Smith, 1989; Read *et al.*, 1991) have suggested that bacteria infecting the bronchial tree are largely associated with intraluminal secretions, rather than adherent to epithelial surfaces. Therefore a high concentration of antibiotic in sputum is desirable. However the penetration of many antibiotic classes into secretions is poor (Bergogne-Berezin, 1988; Valcke, Pauwels & Van der Straeten, 1990). Although the penetration of clarithromycin into lung tissue has been measured, its penetration into secretions has not. We have therefore performed an open study of serum and sputum concentrations of clarithromycin and 14-hydroxy-clarithromycin following single oral administration of 250 and 500 mg of clarithromycin in patients with idiopathic bronchiectasis.

Materials and methods

Patient recruitment and specimen collection

Patients with bronchiectasis confirmed by high resolution thin-cut computerized tomography were enrolled at a time when their symptoms were stable, there having been no infective exacerbation within the last 30 days. Patients who regularly produced mucoid sputum were selected. Nine patients were enrolled during the study period. One patient was withdrawn from analysis because insufficient sputum was obtained. Analysis was performed on eight patients (five male and three female; mean age 53·3 years, S.D. 13·04, range 37–71) who had clinically stable idiopathic bronchiectasis. Mean height was 166·9 cm (S.D. 10·4, range 150·0–182·0) and mean weight was 64·5 kg (S.D. 17·9, range 38–93). All patients had normal liver function tests before entering the trial. No patient had used any concomitant antibiotic, theophylline, carbamazepine, warfarin, digoxin or long-acting (depot) antibiotics within 30 days before the study.

The first dose of clarithromycin (250 or 500 mg) was administered orally following an overnight fast, after physiotherapy to clear residual secretions. Serum and sputum specimens were collected at 0, 1, 2, 4, 8, 24 and 0, 1, 4, 8 and 24 h after administration respectively. At least six days were allowed before the patients returned for their second dose of clarithromycin (250 or 500 mg) when specimens were collected similarly. Fresh sputum specimens were collected by a physiotherapist who provided assistance with postural drainage. Immediately after collection, serum was obtained by centrifugation of blood at 1000 **g** for 5 min at 4°C. Sputum and serum specimens were stored in sterile containers at −70°C. Patients provided written informed consent, and the study was approved by the Ethics Committee of the Royal Brompton National Heart and Lung Hospital.

Analysis of specimens

The concentrations of clarithromycin and 14-hydroxy-clarithromycin in serum and sputum (sol phase) specimens were analysed using high performance liquid chromatography. The apparatus consisted of a pump LKB 2248 with pulse dampener (Pharmacia, Freiburg, Germany); autosampler SIL-8A (Shimadzu Europe, Duisburg, Germany); column heater TCM 100 (30°C, Millpore Waters Chromatography, Eschborn, Germany); electrochemical detector (Coulochem model 5100A: guard cell 5020 set to 1 V, analytical cell 5010 with electrode 1 set to 0·65 V and electrode 2 (for quantitation) set to 0·85 V) (ESA Inc., Bedford, MA, USA); and integrator C-R4A (Shimadzu Europe, Duisburg, Germany). Separation was conducted using a Zorbax

SB-CN column (i.d. 4·6 × 150 mm). The mobile phase consisted of 450 mL 50 mM sodium dihydrogen phosphate, 300 mL acetonitrile and 50 mL methanol, adjusted to pH 7·3 with 10 *N* sodium hydroxide. Using a flow rate of 1 mL/min, the retention times of the compounds to be analysed were 7·7 min (14-hydroxy-clarithromycin), 13·1 min (clarithromycin) and 14·7 min (roxithromycin, internal standard).

Serum

To 500 μL serum were added: 100 μL water (standards: 100 μL water containing 1 μg clarithromycin and 0·5 μg 14-hydroxy-clarithromycin), 100 μL internal standard solution (10 μg/mL roxithromycin in water) and 500 μL acetonitrile for precipitation of proteins. Mixing was followed by incubation at 4°C for 20 min and centrifugation. The supernatant was diluted with 1 mL water and transferred into a disposable column (ADSORBEX^R filled with 100 mg C18 silicagel, Merck Company; Darmstadt, Germany) which had been equilibrated with 5 mL of methanol followed by 2 mL of 50 mM sodium phosphate buffer (pH = 6·3). After the supernatant had passed through the extraction column, it was washed twice with 2·5 mL of 50 mM phosphate buffer (pH = 6·3). The compounds to be investigated were eluted with 1 mL methanol which was subsequently diluted with 1 mL water and then 50 μL was injected into the analytical column.

Sputum

An aliquot of the sputum was adsorbed on to a dental tampon and then transferred to a SALIVETTE^R (Sarstedt Company, Nuembrecht, Germany) and centrifuged (1500 **g**, 20 min). Up to 500 μL of the sol phase fluid was treated in the same manner as serum. The methanol eluate from the extraction column was dried under vacuum in a EVAPOTEC^R Vortex-Evaporator (Haake Buchler Company, Saddle Brook, NJ, USA). The residue was reconstituted in 300 μL of mobile phase and vortex-mixed for 5 sec. An aliquot of 10–20 μL was injected into the analytical column.

Calculation of pharmacokinetic parameters

For each patient, C_{max} was determined as the highest concentration of clarithromycin or 14-hydroxy-clarithromycin achieved in serum or sputum for each dosage of clarithromycin. A mean C_{max} was calculated for clarithromycin or 14-hydroxy-clarithromycin as the mean of the individual C_{max} values obtained from each of the eight patients. Similarly mean T_{max} was determined for clarithromycin and 14-hydroxy-clarithromycin in sputum and serum for each dosage of clarithromycin. The sputum to serum ratio for clarithromycin and 14-hydroxy-clarithromycin was determined for each individual time point as the percentage ratio of sputum concentration of clarithromycin or 14-hydroxy-clarithromycin to that of a simultaneous serum sample. The elimination constants (k_{el}) for clarithromycin and 14-hydroxy-clarithromycin in serum were calculated from the terminal part of the $\log_e$ serum concentration versus time curve. The half-lives of clarithromycin and 14-hydroxy-clarithromycin were calculated by using the formula $T_{1/2} = -0{\cdot}6931/k_{el}$ (Clark & Smith, 1986).

Table I. Serum concentrations of clarithromycin and 14-hydroxy-clarithromycin after a single oral dose of 250 mg and 500 mg clarithromycin

Dose time (h)	250 mg CL		500 mg CL	
	CL (mg/L)	HCL (mg/L)	CL (mg/L)	HCL (mg/L)
0	0±0	0±0	0±0	0±0
1	0·76±0·64	0·20±0·18	2·11±1·75	0·55±0·46
2	0·93±0·59	0·31±0·16	2·65±1·43	0·56±0·38
4	0·75±0·42	0·28±0·10	2·12±1·02	0·57±0·36
8	0·54±0·34	0·23±0·08	1·31±0·57	0·45±0·24
24	0·09±0·09	0·07±0·06	0·28±0·24	0·14±0·10

All data are means of eight patients±standard deviation. CL, Clarithromycin; HCL, 14-hydroxy-clarithromycin.

Calibration and control samples

Internal standardization using roxithromycin and quality control was performed by assaying spiked samples with each run. The detection limit was 400 pg for clarithromycin and 14-hydroxy-clarithromycin and 600 pg for roxithromycin.

Statistical analysis

A repeated measure analysis of variance was undertaken to determine differences in the mean concentration between drug doses and whether there was a change in concentration with time. The C_{max} of sputum and serum were compared by Wilcoxon signed rank test. A P value of less than 0·05 was taken as significant. All analyses were performed with the aid of the statistical package BMDP (BMDP, 1990).

Results

Serum concentrations (Table I)

The mean concentrations of clarithromycin and 14-hydroxy-clarithromycin were significantly higher in the 500 mg group than in the 250 mg group ($P < 0·05$). The concentrations of clarithromycin and 14-hydroxy-clarithromycin increased until C_{max} was reached, thereafter decreasing with time. As there were relatively few time points for sampling, half-lives calculated are very variable and there is no evidence of any difference in the half-lives calculated for both doses (Table III). The C_{max} for clarithromycin (250 mg) and 14-hydroxy-clarithromycin were 1·20±0·39 mg/L and 0·37±0·10 mg/L respectively, which occurred at 3 and 3·1 h. The C_{max} of clarithromycin (500 mg) and 14-hydroxy-clarithromycin were 2·78±1·31 mg/L and 0·68±0·36 mg/L respectively, which occurred at 2·5 and 2·6 h, and were significantly higher than those following clarithromycin (250 mg) ($P < 0·05$).

Sputum concentrations (Table II)

The mean concentrations of clarithromycin ($P < 0·01$) and 14-hydroxy-clarithromycin ($P < 0·05$) were significantly higher in the 500 mg group than in the 250 mg group. The concentrations of clarithromycin and 14-hydroxy-clarithromycin increased until C_{max}, thereafter decreasing with time. Sputum half-lives were not calculated in view of the

Table II. Sputum concentrations of clarithromycin and 14-hydroxy-clarithromycin in patients following a single oral dose of 250 mg and 500 mg clarithromycin

Dose time (h)	250 mg CL		500 mg CL	
	CL (mg/L)	HCL (mg/L)	CL (mg/L)	HCL (mg/L)
0	0±0	0±0	0±0	0±0
4	0·43±0·34	0·24±0·26	1·49±1·02	0·43±0·25
8	0·44±0·23	0·26±0·16	0·91±0·39	0·33±0·13
24	0·10±0·07	0·05±0·05	0·37±0·29	0·17±0·16

All data are means of eight patients ± standard deviation. CL, Clarithromycin; HCL, 14-hydroxy-clarithromycin.

limited data. The C_{max} of clarithromycin (250 mg) and 14-hydroxy-clarithromycin in sputum were 0·52±0·30 and 0·30±0·24 mg/L respectively which occurred at 5 h and 6·5 h (Table III). The C_{max} of clarithromycin (500 mg) and 14-hydroxy-clarithromycin were 1·59±1·00 mg/L and 0·47±0·23 mg/L respectively which occurred at 5 and 5·5 h. At C_{max}, only sputum clarithromycin concentration was significantly higher following 500 mg clarithromycin compared with 250 mg clarithromycin ($P < 0·05$). The sputum/serum percentage ratios at C_{max} (sputum) for clarithromycin and 14-hydroxy-clarithromycin were 74·3% and 113·9% (250 mg clarithromycin) and 94·7% and 99·9% (500 mg clarithromycin) respectively.

Discussion

These results confirm previously published reports that orally administered clarithromycin is rapidly absorbed and elimination from serum is slow (Ferrero, 1990; Davey, 1991). Peak serum concentrations were achieved 3 h after oral administration of 250 and 500 mg of clarithromycin, while peak sputum concentrations were achieved after 5 h. In this study, the mean serum concentrations of clarithromycin and 14-hydroxy-clarithromycin are comparable to results of studies performed on healthy individuals (Ferrero, 1990; Davey, 1991).

Measurement of antibiotic penetration into the lung is difficult because of the heterogeneity of tissue demonstrated in studies of bronchial biopsies (Baldwin *et al.*, 1992) and lung parenchyma tissue obtained at thoracotomy, which are often contaminated with blood (Valcke *et al.*, 1990). Antibiotic concentrations in epithelial lining fluid can be obtained by bronchoalveolar lavage (Baldwin *et al.*, 1991) and contamination minimized by microlavage, but may not be representative of those in bronchial mucus. Similarly, bronchial mucus can only be obtained at bronchoscopy, which must be repeated for pharmacokinetic studies. The use of sputum is convenient but is also complicated by contamination with saliva, stagnant mucus (i.e. present before antibiotic was taken) and β-lactamase; furthermore, it may be difficult to obtain adequate fresh specimens (Pennington, 1981). In this study, the choice of stable bronchiectasis patients who regularly produced mucoid sputum, and expectoration assisted by physiotherapists may have overcome many of these problems.

The concentration of antibiotic at the site of infection may be more important than serum concentrations in patients with bronchial infections. Recent in-vivo and in-vitro studies have shown that bacteria are associated with damaged epithelium, and parti-

Table III. Pharmacokinetic data for clarithromycin (CL) and 14-hydroxy-clarithromycin (HCL) following a single oral dose.

	250 mg CL				500 mg CL			
	serum CL	sputum CL	serum HCL	sputum HCL	serum CL	sputum CL	serum HCL	sputum HCL
C_{max} (mg/L)	1·2 ± 0·39 (0·7–1·89)	0·52 ± 0·3 (0·23–1·14)	0·37 ± 0·1 (0·25–0·5)	0·3 ± 0·24 (0·15–0·86)	2·78 ± 1·31 (1·07 ± 5·59)	1·59 ± 1·0 (0·62–3·49)	0·68 ± 0·36 (0·19–1·44)	0·47 ± 0·23 (0·12–0·85)
T_{max} (h)	3·0 ± 2·3 (1–8)	5·0 ± 1·9 (4–8)	3·1 ± 2·2 (1–8)	6·5 ± 2·1 (4–8)	2·5 ± 2·3 (1–8)	5·0 ± 1·9 (4–8)	2·6 ± 2·6 (1–8)	5·5 ± 2·1 (4–8)
$T_{1/2}$ (h)	8·0 ± 4·9 (3·4–18·2)	— —	10·1 ± 4·5 (3·6–16·4)	— —	6·9 ± 3·0 (4·3 ± 11·6)	— —	12·4 ± 15·0 (3·6 ± 46·1)	— —

Data are means ± S.D. and range obtained from eight patients.

cularly with intraluminal secretions in the lower respiratory tract (Farley *et al.*, 1986; Baltimore *et al.*, 1989; Read *et al.*, 1991). Therefore penetration into these sites may influence the efficacy of an antibiotic. Many antibiotics, particularly the penicillins, have poor penetration into bronchial secretions (Bergogne-Berezin, 1988; Valcke *et al.*, 1990). In the present study, the the penetration of clarithromycin and 14-hydroxy-clarithromycin into sputum was rapid and the concentrations achieved were higher than those previously reported for β-lactam antibiotics. Drug elimination of clarithromycin and 14-hydroxy-clarithromycin from sputum was slow and measurable concentrations of antibiotics were still present in sputum 24 h after a single oral dose of clarithromycin. For both doses of clarithromycin administered, the sputum to serum ratio of clarithromycin ranged from 31·9% to 257·8% and 14-hydroxy-clarithromycin ranged from 40·4% to 277·9%. Clarithromycin has also been shown to have good penetration into lung tissue (C_{max} lung tissue 17·47 mg/kg and C_{max} serum 2·82 mg/L) following multiple doses of 500 mg taken orally (Fraschini *et al.*, 1991). Previous studies on erythromycin generally used multiple doses of erythromycin and have shown that the penetration of erythromycin ranges from 10% to 50% for bronchial secretions and sputum (Marlin *et al.*, 1980; Bergogne-Berezin, 1988; Periti *et al.*, 1989), 83% for bronchial mucus (Brun *et al.*, 1981), and 110% to 320% for lung parenchyma (Wollmer *et al.*, 1982). Our results indicate that clarithromycin has a higher penetration into respiratory mucus than erythromycin. Further studies are needed to determine whether clarithromycin and 14-hydroxy-clarithromycin accumulate in sputum after multiple doses.

The mean sputum concentrations of clarithromycin and 14-hydroxy-clarithromycin were compared with the MICs for common respiratory pathogens (King & Phillips, 1991; Piscitelli, Danziger & Rodvold, 1992). After administration of the two doses of clarithromycin, sputum concentrations of clarithromycin and 14-hydroxy-clarithromycin exceeded the MICs for *Streptococcus pneumoniae* (0·12 mg/L for clarithromycin 0·015 mg/L for 14-hydroxy-clarithromycin), *Moraxella catarrhalis* (0·12 mg/L, 0·12 mg/L), and *Streptococcus pyogenes* (0·06 mg/L, 0·03 mg/L), for more than 24 h, except following 250 mg doses, when the MIC for *Streptococcus pneumoniae* (clarithromycin) was exceeded for 21·5 h and *Moraxella catarrhalis* when the MIC was exceeded for 21·5 h (clarithromycin) and 16·5 h (14-hydroxy-clarithromycin). MICs for *Haemophilus influenzae* vary considerably: 2–16 mg/L in one study, 0·75–4 mg/L in another (Dabernat *et al.*, 1991; Olsson-Liljequist & Hoffman, 1991). However, the MIC for *H. influenzae* was not exceeded in sputum following either single dose of clarithromycin. Multiple doses of clarithromycin are known to increase the serum concentrations of clarithromycin, which may be reflected in sputum concentrations. Although sputum concentrations of clarithromycin and 14-hydroxy-clarithromycin are below the MIC for *H. influenzae*, clarithromycin has been shown to be effective in treatment of patients with infective exacerbation of chronic bronchitis caused by *H. influenzae* (Guay & Craft, 1992). The recent finding that *H. influenzae* infection caused less tissue damage in the presence of subinhibitory antibiotic concentrations may be relevant in these circumstances (Tsang *et al.*, 1993).

Macrolide antibiotics have been used more frequently of late because of recognition of infections caused by bacteria not sensitive to β-lactams, such as mycoplasma, legionella and chlamydia. Clarithromycin has good sputum penetration in patients with clinically stable bronchiectasis. This property, and its superior pharmacokinetic profile, give it clear advantages over older macrolide antibiotics such as erythromycin.

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