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Comparative distribution of azithromycin in lung tissue of patients given oral daily doses of 500 and 1000 mg

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***Objectives*: The administration of antibacterial agents should be optimized on the basis of their distribution to enhance drug exposure and obtain bacterial eradication. This study examines the pharmacokinetics of azithromycin in plasma, lung tissue and bronchial washing in patients after oral administration of 500 mg versus 1000 mg once daily for 3 days.**

***Patients and methods*: Samples of plasma, lung tissue and bronchial washing were obtained from a cohort of 48 patients during open-chest surgery for lung resection up to 204 h after the last drug dose, and assayed for antibiotic concentrations.**

***Results*: Azithromycin was widely distributed within the lower respiratory tract and sustained levels of the drug were detectable at the last sampling time in lung tissue. Doubling the dose of the antibiotic resulted in a proportional increase in lung area under the curve (AUC, 1245.4 versus 2514.2 h × mg/kg) and peak tissue concentration (C_{max}, 8.93 ± 2.05 versus 18.6 ± 2.20 mg/kg). The pharmacodynamic parameter AUC/MIC for susceptible and intermediate strains of *Streptococcus pneumoniae* (MICs 0.5 and 2 mg/L, respectively) increased after administration of the 1000 mg schedule compared with 500 mg ($AUC/MIC_{0.5}$ 2414 versus 1144 and AUC/MIC_2 2112 versus 814.1 h × mg/kg, respectively) in pulmonary tissue.**

***Conclusions*: Lung exposure to azithromycin is increased proportionally by doubling the dose, which results in a predictable pharmacokinetic behaviour of the drug in the lower respiratory tract.**

Keywords: pharmacokinetics, microbiological assay, azalide, *Streptococcus pneumoniae*, pharmacodynamics

Introduction

Azithromycin is an advanced-generation azalide antibiotic that contains a nitrogen atom in the macrolide aglycone ring. The drug is active *in vitro* against *Streptococcus pneumoniae*, group A streptococci, *Streptococcus agalactiae*, *Staphylococcus aureus*, *Haemophilus influenzae*, *Moraxella catarrhalis* and intracellular organisms such as *Chlamydia*, *Mycoplasma* and *Legionella* spp.[1,2] Azithromycin retains the Gram-positive activity of erythromycin, as a result of a common mechanism of action, but provides enhanced activity against Gram-negative aerobes. The pharmacokinetics of azithromycin are characterized by rapid and extensive uptake within the intracellular compartment, with high and sustained antibiotic concentrations in tissues.[3] To a greater extent than other macrolides, azithromycin is concentrated into phagocytic cells;[4–6] therefore, its ability to reduce the viability of intracellular bacteria is enhanced. The low plasma concentrations of azithromycin do not adversely influence its efficacy, since the activity of azithromycin in models of respiratory tract

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infections correlates with pulmonary antibiotic levels, even though plasma concentrations remain below the MIC for the infecting organism.[7]

S. pneumoniae is the most significant microorganism involved in respiratory tract infections in outpatients, on the basis of epidemiological data and the rates of morbidity and mortality associated with this pathogen.[8,9] Current treatment of infections by *S. pneumoniae* consists of the administration of β-lactams, fluoroquinolones or macrolides/azalides;[9] in particular, azithromycin is given at various schedules, including 500 mg once daily for 3 days[10] or 500 mg on day 1 followed by 250 mg on days 2–5, providing sustained drug concentrations at sites of infection for up to 10 days.[11] Azithromycin is an effective agent for the management of community-acquired pneumonia;[12] however, the emergence of strains of *S. pneumoniae* with reduced susceptibility to macrolides[8] poses the question of whether increasing the drug dose may result in enhanced tissue uptake and improved antibacterial efficacy. Indeed, the safety profile of azithromycin may allow the investigation of alternative treatment schedules, provided that the pharmacokinetic profile of the drug justifies this approach. Therefore, the present study compares for the first time the antibiotic concentrations in plasma, bronchial washing and lung parenchyma after a 3 day, once-daily oral regimen of the standard 500 mg dose versus 1000 mg administered to patients undergoing surgery for lung resection, to ascertain whether the administration of the higher dosage produces a predictable increase in tissue antibiotic levels and a pharmacokinetic advantage to be exploited in the clinical setting.

Materials and methods

Study design

This open-label, randomized trial was approved by the Ethics Committee of Pisa University Hospital. Patients affected by lung cancer, requiring open-chest surgery for lung resection were eligible; they were advised of the investigational nature of this study and provided written informed consent. Conditions for study enrolment were: (a) history of cigarette smoking of less than five pack-years; (b) absence of known hypersensitivity or intolerance to macrolide antibiotics; (c) no administration of an investigational drug within 4 weeks before enrolment; (d) adequate renal (serum creatinine ≤ 132 μmol/L) and hepatic function (serum bilirubin level ≤ 34 μmol/L and serum aspartate aminotransferase/alanine aminotransferase level ≤2 times the upper limit of normal); (e) absence of conditions affecting drug absorption (i.e. gastrectomy, short bowel syndrome, chronic pancreatitis, protein-losing enteropathy, steatorrhoea, Whipple's disease, coeliac sprue, vomiting/diarrhoea); (f) no drug or alcohol dependence; (g) absence of diffuse, non-infectious lung diseases (i.e. sarcoidosis, fibrosis); (h) no antibiotic prophylaxis before surgery; and (i) no administration of drugs known to interfere with azithromycin pharmacokinetics.[13] Each subject gave a medical history and underwent physical examination and baseline laboratory testing (urinalysis, complete blood count and platelet count, creatinine and serum concentrations of AST/ALT, alkaline phosphatase, total and fractionated bilirubin, total protein and albumin, lactate dehydrogenase, calcium, phosphorus, glucose and electrolytes) within 1 week before drug administration and during hospitalization for surgery. Laboratory tests were repeated after surgery as a routine procedure, not protocol-directed.

Azithromycin (500 mg capsules, Pfizer, Italy) was administered by oral route to two groups of 24 subjects each, following an investigator-generated randomization scheme: 16 males and eight females (age range 36–70 years, median age 64 years) were given azithromycin 500 mg once daily for three consecutive days, whereas 18 males and six females (age range 35–69 years; median age 61 years) received azithromycin at the 1000 mg dose level. The drug was taken at 8 a.m. after an 8 h fasting period and subjects continued fasting for 2 h after each dose. Patients were not allowed to recline or drink beverages containing caffeine for 2 h post-dose, and low-fat meals were provided during drug administration. Treatment tolerability was assessed on the basis of adverse events reported by patients and laboratory tests on blood and urine.

Sample collection and processing

Peripheral venous blood (4 mL) was obtained from all subjects before drug administration and 1, 4, 6, 12, 24, 48, 60, 72, 96, 108, 120, 156 and 204 h thereafter; samples were placed in heparinized tubes and centrifuged at 2500 rpm for 15 min to separate plasma. Specimens of lung and bronchial washing were collected 6, 12, 60, 108, 156 and 204 h after the last dose of azithromycin from four patients per time point. Because of the long half-life of the drug, a maximum deviation of 15 min before or after the indicated time points for tissue and bronchial washing collection was allowed. Bronchial washing was carried out by a flexible bronchoscope inserted into the endotracheal tube to deliver three aliquots (20 mL each) of pre-warmed sterile 0.9% NaCl into the right middle lobe. Each aliquot was immediately aspirated and a volume of 15.6 ± 2.1 mL (mean ± S.D.) was recovered; the first aspirate was discarded to avoid contamination with proximal airway fluids and cells, whereas the second and third were pooled. The average duration of the procedure was 2 min. Bronchial washings were spun at 2500 rpm for 15 min to remove cells. Multiple specimens (n = 4, ~0.5 g each) of macroscopically normal lung were obtained from each patient at least 10 mm from the pathological tissue. Samples were briefly rinsed in phosphate-buffered saline (pH 7.4) and blotted with a sterile gauze, moistened with saline to remove excess blood and finally weighed. Microscopic examination of each specimen

was carried out before the antibiotic assay to confirm normal pulmonary structure; otherwise, the abnormal tissue was rejected. All specimens were frozen at –80°C for no longer than 2 weeks before drug analysis.

Azithromycin assay

Azithromycin concentration was measured by agar diffusion bioassay, using *Micrococcus luteus* NCTC 8440 as the indicator organism.[14] The assay was linear in the range of 0.01–35 mg/L for plasma and bronchial washing and 0.02–35 mg/kg for pulmonary tissue; calibration curves yielded a correlation coefficient better than 0.99. Method validation was as follows: intra-assay precisions ranged from 3.5% to 6.5%, and inter-assay precision, at a level of 0.12 mg/L and 0.12 mg/kg, ranged from 3.8% to 6.7%. Mean recovery of azithromycin from plasma and bronchial washing was 96%, whereas from lung it was 94%.

Pharmacokinetic and pharmacodynamic study

Pharmacokinetics of azithromycin were estimated by non-compartmental analysis.[15] For each subject, the apparent C_{max} (mg/L or mg/kg) and T_{max} (h) of azithromycin in plasma, lung and bronchial washing were determined by visual inspection of the concentration versus time curves. The terminal $t_{1/2}$ of azithromycin was calculated for plasma as $0.693/k_{el}$; k_{el} (1/h) was obtained from linear regression analysis of the terminal portion of the semi-logarithmic plasma concentration versus time curve. The AUC (h × mg/L or h × mg/kg) from time zero to 204 h (or last detectable concentration) was determined for plasma, bronchial washing and pulmonary tissue by the linear trapezoidal rule. Since patients were assigned to a specific time for collection of lung specimens and bronchial washing, only one concentration/time point of azithromycin was obtained from each subject. Therefore, the AUCs of bronchial washing and lung were calculated on the mean concentration versus time data, as carried out in previous studies;[16–18] on the other hand, the AUC values for plasma were calculated on individual drug level versus time curves. Additional pharmacokinetic parameters calculated on plasma were the apparent volume of distribution (V_{app}, L), calculated as dose/AUC_{plasma} × k_{el}, the apparent oral systemic clearance (CL_{app}, L/h), obtained as $V_{app} \times k_{el}$, which is equivalent to dose/AUC_{plasma}. Penetration ratios of azithromycin in pulmonary tissue and bronchial washing were determined as AUC_{lung}/AUC_{plasma} and $AUC_{bronchial\ washing}/AUC_{plasma}$. Finally, pharmacodynamic parameters were calculated, where applicable, for plasma, bronchial washing and lung, as follows: (a) the area under the inhibitory curve (AUC/MIC, h × mg/L or h × mg/kg), the trapezoidal AUC diminished by the area below the MIC; and (b) the $T > MIC$ (h), the time that azithromycin concentrations remained above the MIC, which was calculated as the difference between the time point at which the antibiotic concentration equals the MIC for the first time following drug administration, and the time point at which the antibiotic concentration equals the MIC for the last time after azithromycin administration.[3,19] Pharmacodynamic parameters were calculated on the basis of the breakpoint MIC for susceptible and intermediate strains of *S. pneumoniae* (MIC 0.5 and 2 mg/L, respectively).[20]

Statistical analysis

Results are given as means ± S.D. Two-tailed Student's *t*-test for unpaired data was used to evaluate the statistical difference between pharmacokinetic parameters and significance was defined as $P < 0.05$.

Results

The main pharmacokinetic and pharmacodynamic parameters of azithromycin are reported in Tables 1 and 2, and the profiles of azithromycin concentration versus time in plasma, bronchial washing and lung tissue at 500 and 1000 mg once

Table 1. Pharmacokinetic parameters of azithromycin in plasma of 48 patients

	Azithromycin 500 mg once daily for 3 days	Azithromycin 1000 mg once daily for 3 days
C_{max} (mg/L)	0.18 ± 0.06	0.32 ± 0.08[a]
T_{max} (h)	12	12
AUC (h × mg/L)	11.62 ± 3.8	19.83 ± 2.9[a]
k_{el} (1/h)	0.01803 ± 0.004	0.01555 ± 0.003
$t_{1/2}$ (h)	38.45 ± 6.1	44.58 ± 6.8
CL_{app} (L/h)	43.03 ± 7.2	50.48 ± 8.7
V_{app} (L)	2386.6 ± 375.3	3246.7 ± 618.5

Each value is the mean ± S.D. of 24 patients.
[a] $P < 0.05$ compared with azithromycin 500 mg once daily for 3 days.

Table 2. Pharmacokinetic and pharmacodynamic parameters of azithromycin in lung and bronchial washing of 48 patients

	Azithromycin 500 mg once daily for 3 days		Azithromycin 1000 mg once daily for 3 days	
	bronchial washing	lung	bronchial washing	lung
Pharmacokinetics				
C_{max} (mg/L or mg/kg)	0.83 ± 0.07	8.93 ± 2.05	1.49 ± 0.09[a]	18.6 ± 2.20[a]
T_{max} (h)	12	60	12	60
AUC (h × mg/L or h × mg/kg)	70.29	1245.4	139.9	2514.2
$AUC_{bronchial\ washing}/AUC_{plasma}$	6.05	–	7.06	–
AUC_{lung}/AUC_{plasma}	–	107.2	–	126.8
Pharmacodynamics[b]				
$AUC/MIC_{0.5}$ (h × mg/L or h × mg/kg)	11.1	1144	41.55	2414
AUC/MIC_2 (h × mg/L or h × mg/kg)	–	814.1	–	2112
$T > MIC_{0.5}$ (h)	48	>204	102	>204
$T > MIC_2$ (h)	–	150	–	198

C_{max} and T_{max} are the mean ± S.D. of four patients.
[a]$P < 0.05$ compared with azithromycin 500 mg once daily for 3 days.
[b]Pharmacodynamic parameters are calculated on the basis of the MIC for azithromycin-susceptible (0.5 mg/L) or -intermediate (2 mg/L) *S. pneumoniae*.

daily for 3 days are shown in Figure 1. A significant increase was observed at 500 mg versus 1000 mg treatment schedule in plasma C_{max} (0.18 ± 0.06 versus 0.32 ± 0.08 mg/L, $P < 0.05$) and AUC (11.62 ± 3.8 versus 19.83 ± 2.9 h × mg/L, $P < 0.05$; Table 1). The apparent T_{max} was the same in plasma and bronchial washing (12 h), but was delayed in the lung (60 h). The $t_{1/2}$ of azithromycin in plasma showed modest differences between the 500 and 1000 mg drug schedules (38.45 versus 44.58 h, Table 1). Furthermore, the values of plasma CL_{app} at 500 versus 1000 mg showed minor changes (43.03 ± 7.2 versus 50.48 ± 8.7 L/h, Table 1), suggesting the lack of saturation of drug elimination from the body. Finally, the large V_{app} is in agreement with the wide distribution and uptake of azithromycin in peripheral tissue compartments.

A proportional and significant increase was shown in azithromycin C_{max} in bronchial washing (0.83 ± 0.07 versus 1.49 ± 0.09 mg/L, $P < 0.05$) and lung (8.93 ± 2.05 versus 18.6 ± 2.20 mg/kg, $P < 0.05$) after administration of standard versus double drug dose (Table 2). The AUC of azithromycin in pulmonary tissue increased proportionally, suggesting that tissue reservoirs were able to uptake the antibiotic at the higher dosage of 1000 mg/daily. As expected, azithromycin was preferentially distributed in bronchial washing ($AUC_{bronchial\ washing}/AUC_{plasma}$, 6.05 and 7.06), as well as in lung tissue (AUC_{lung}/AUC_{plasma}, 107.2 and 126.8) at 500 and 1000 mg, respectively (Table 2). Finally, the increase in drug dose was associated with a proportional increment in the AUC/MIC of azithromycin in pulmonary tissue (Table 2).

No serious adverse events were reported; mild gastrointestinal discomfort and loose stools, not requiring discontinuation of azithromycin administration, were noticed by a few patients, without differences between the 500 mg (6/24 patients) and 1000 mg (7/24 patients) treatment groups. Routine serum chemistry and urinalysis carried out before and during the study period demonstrated no significant changes with respect to baseline.

Discussion

Several pharmacokinetic/pharmacodynamic parameters, e.g. AUC/MIC and $T > MIC$, have been examined for antibiotics, in order to optimize the treatment on the basis of drug distribution and antimicrobial activity.[3,19] The analysis of drug concentration in plasma is appropriate for conditions in which the central compartment is the main site of disease, e.g. septicaemia or endocarditis, whereas, for infections localized in the extravascular compartments, e.g. respiratory tract, tissue antibiotic concentrations are more informative. Most studies have investigated the pharmacokinetic profile of azithromycin after 500 mg single dose[16] or multiple doses of 500 mg on day 1 and 250 mg on days 2–5,[5,6,11] whereas the present study was the first to investigate whether the increase in the drug dose to 1000 mg daily for 3 days resulted in a pharmacokinetic advantage that could be exploited to improve the efficacy of azithromycin in the treatment of respiratory tract infections.

From a microbiological point of view, *S. pneumoniae* is the most common cause of community-acquired pneumonia, followed by atypical pathogens, including *Mycoplasma pneumoniae*.[21] Azalides are among the recommended agents for first-line therapy for adults with community-acquired pneumonia, and they have the advantage of convenient

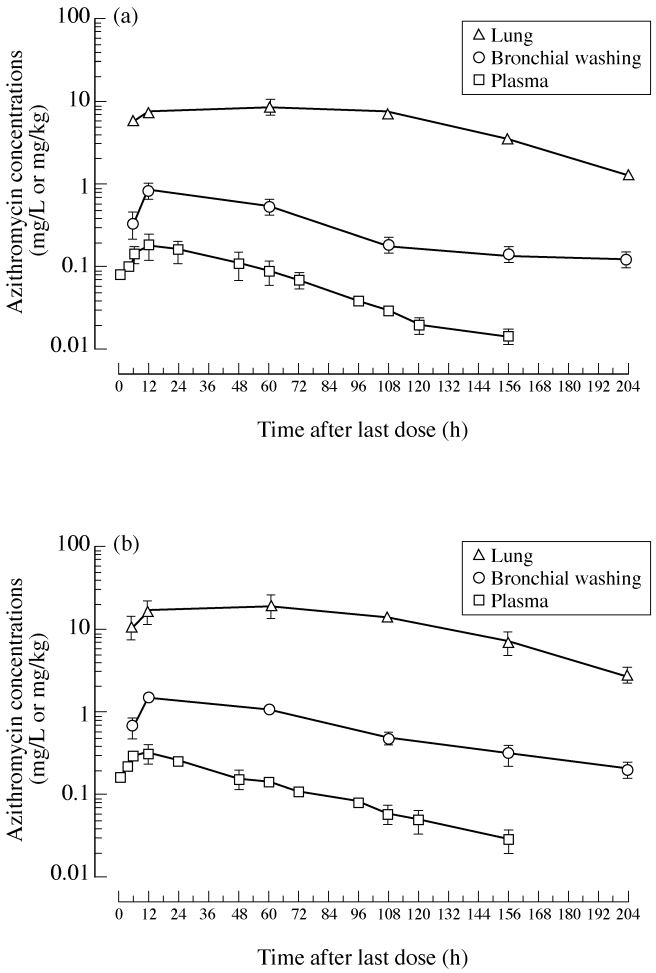

Figure 1. Comparative distribution of azithromycin in lung tissue of patients given oral daily doses of 500 and 1000 mg. Concentrations versus time semi-logarithmic plot of azithromycin 500 mg (upper) and 1000 mg (lower) once daily for 3 days in lung tissue, bronchial washing and plasma. Points, mean values of 24 patients (plasma) or four patients (bronchial washing and lung tissue); vertical bars, S.D. If not shown, S.D. is within the symbol size.

dosing schedules and good tolerability profiles; however, reduction in susceptibility to macrolides among respiratory pathogens is steadily increasing in the USA and Europe.[21–23] The modification of the schedule of treatment is unlikely to be able to suppress the proliferation of highly-resistant strains [i.e. *S. pneumoniae* with the MLS_B phenotype associated with expression of the *erm*(AM) gene] and characterized by an erythromycin MIC > 64 mg/L.[24,25] However, the resistance to macrolides (i.e. erythromycin MICs 2–8 mg/L) of *S. pneumoniae* strains characterized by the M phenotype and dependent on the expression of the *mef*(E) gene, which encodes a macrolide efflux pump,[26] may be overcome by the increase in antibacterial drug dose.[9] However, as a result of the high level of tissue uptake of azithromycin, the strategy of dose increase to enhance its therapeutic efficacy may be acceptable provided that the drug pharmacokinetics behave linearly with proportional and predictable increase in tissue levels at the higher dose of 1000 mg once daily for 3 days, since peripheral reservoirs might already be saturated at the lower 500 mg dose level.

The present experimental findings provide evidence that enhanced pulmonary tissue exposure may be obtained by the administration of azithromycin 1000 mg for 3 days. In particular, the increase in drug dose was associated with at least a doubling in the main pharmacokinetic and pharmacodynamic parameters, particularly C_{max}, AUC and AUC/MIC. Furthermore, the penetration ratio of azithromycin in lung tissue was high in both groups of patients, as demonstrated by the AUC_{lung}/AUC_{plasma} ratio, suggesting that tissue reservoirs of azithromycin were not saturated at the conventional 500 mg drug dose and additional antibiotic may reach the sites of infection.

In this study, the concentrations of azithromycin in plasma were lower than in bronchial washing and the difference was most evident if these data were compared with drug distribution into the lung. This behaviour may be explained by the extensive uptake of the antibiotic by pulmonary tissue from which it can be released into bronchial secretions by cells, particularly macrophages and fibroblasts, in which azithromycin is highly concentrated in lysosomes.[27,28] The highly preferential cellular distribution of the drug provides an explanation for the difference observed between the present levels of azithromycin in lung tissue, which contains acellular stroma, and the higher concentrations of the antibiotic in alveolar macrophages,[5,6,11] whereas plasma and epithelial lining fluid levels of azithromycin of previous studies were comparable to the present data. The investigation of the cellular distribution of azithromycin was not the object of the present study, since the preferential uptake of the drug by monocyte/macrophages, granulocytes[29,30] and fibroblasts[27,31] has been widely documented in the scientific literature. Indeed, tissue kinetics of azithromycin is characterized by a complex turnover, in which fibroblasts and macrophages play an important role. In particular, intracellular penetration of azithromycin in fibroblasts occurs rapidly and then increases progressively over a 3 day period; once concentrated in fibroblasts, azithromycin is released slowly and, after incubation for 48 h in drug-free medium, 63% of the initial amount of azithromycin is released by cells, independently of exposure to bacteria.[27] The widespread distribution of fibroblasts in tissues, including lung, suggests a potential role for these cells, and possibly other lysosome-containing tissue cells, to serve as a reservoir for azithromycin, slowly releasing it for activity against extracellular organisms and passing it to phagocytes for activity against intracellular pathogens and potential transport to sites of infection.[26,32]

In conclusion, the data from the present study provide evidence that the current treatment of respiratory tract infec-

tions with azithromycin may be improved by appropriate rescheduling because of the favourable pharmacokinetic profile of the drug in the lower respiratory tract observed after administration of three oral daily doses of 1000 mg.

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