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Published in:
Therapeutic Drug Monitoring

DOI:
[10.1097/01.ftd.0000195617.69721.a5](https://doi.org/10.1097/01.ftd.0000195617.69721.a5)

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2006

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Citation for published version (APA):

Wilms, E. B., Touw, D. J., & Heijerman, H. G. M. (2006). Pharmacokinetics of azithromycin in plasma, blood, polymorphonuclear neutrophils and sputum during long-term therapy in patients with cystic fibrosis. *Therapeutic Drug Monitoring*, 28(2), 219-225. <https://doi.org/10.1097/01.ftd.0000195617.69721.a5>

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Pharmacokinetics of Azithromycin in Plasma, Blood, Polymorphonuclear Neutrophils and Sputum During Long-Term Therapy in Patients With Cystic Fibrosis

E. B. Wilms, PharmD,* D. J. Touw, PharmD, PhD,* and H. G. M. Heijerman, MD, PhD†

Abstract: Chronic therapy with the macrolide antibiotic azithromycin (AZM) is widely practiced in the treatment of patients with cystic fibrosis (CF) and chronic lung infection with *Pseudomonas aeruginosa*. Azithromycin dosage is variable, based on published studies, and not supported by pharmacokinetic data. This study describes the pharmacokinetics of the long-term administration of AZM (500 mg per day) in CF patients. AZM concentrations were quantified in the plasma, blood, isolated polymorphonuclear neutrophils (PMNNs), and sputum of 8 adult CF patients. The AZM distribution $t_{1/2}$ was 0.1 hours in plasma. The (mean $\pm$ standard deviation) elimination $t_{1/2}$ was 102 ± 20 hours in plasma, 180 ± 68 hours in blood, and 289 ± 166 hours in PMNNs. The C_{max} of AZM was 0.67 ± 0.31 mg/L in plasma and 2.01 ± 0.74 mg/L in blood, of which 1.44 ± 0.69 mg/L was found in PMNNs. In sputum the concentration of AZM ranged from 12 to 53 mg/L and was still detectable at concentrations in the range 4 to 27 mg/L 10 days after the last dose. On average, the concentration in PMNNs was 2100 times the C_{plasma} 24 hours after dosing AZM. These results confirm the accumulation of AZM in PMNNs. The authors conclude that sputum levels are elevated far above plasma and blood concentrations. The long $t_{1/2}$ in blood and PMNNs and the slow decrease in sputum levels indicate a less frequent dosing schedule (for instance once weekly) should be studied in future clinical trials of AZM in patients with cystic fibrosis.

Key Words: azithromycin, cystic fibrosis, pharmacokinetics, polymorphonuclear neutrophils, sputum concentration, therapeutic drug monitoring

(*Ther Drug Monit* 2006;28:219–225)

Azithromycin (AZM) is a 15-membered ring azalide antibiotic related to the macrolide antibiotics. AZM exhibits a bacteriostatic effect toward susceptible pathogens by interfering with RNA-dependent protein synthesis.¹

AZM is generally administered for short periods of time (usually during 3 consecutive days) (Product Information Zithromax® nr 70-5179-00-2 Pfizer Inc., NY, rev. 08-2003). Accumulation in tissues and white blood cells (both polymorphonuclear neutrophils and monocytes) has been associated with a prolonged effect.^{2,3}

Long-term administration of AZM is able to reduce or stabilize clinical symptoms of airway inflammation in patients chronically infected with *Pseudomonas aeruginosa* in patients with diffuse panbronchiolitis and cystic fibrosis (CF).^{4–7} In vitro, the minimal inhibitory concentration (MIC) of AZM for *P. aeruginosa* is as high as 100 mg/L.⁸ *P. aeruginosa* is considered “resistant” to AZM on the basis of MIC determination. Sub-MIC concentrations (2 mg/L in strain PAO₁), however, did inhibit the formation of factors promoting *P. aeruginosa* biofilm formation (quorum sensing factors).^{8–10} The biofilm protects the bacteria against the immune system of the host and the penetration of antibiotics. Three recent, randomized, clinical trials in patients with CF used dosing schedules ranging from 250 mg 3 times per week to 500 mg once daily. AZM therapy reduced the number of respiratory exacerbations, improved lung function, or reduced the rate of decline in lung function.^{4,6,7}

To date, no pharmacokinetic studies have been published to support long-term dosing schedules of AZM in patients with CF. An optimal dosing schedule for chronic administration of AZM in pulmonary infections caused by *P. aeruginosa* remains unclear.

In pharmacokinetic studies after short-term use of AZM in healthy subjects/volunteers, it was found that plasma concentrations decline rapidly ($t_{1/2} = 3$ hours) during the distribution phase; however, in the elimination phase low plasma concentrations could be detected for an extended period ($t_{1/2} = 30–93$ hours).^{11–14} In isolated polymorphonuclear neutrophils (PMNNs), accumulation of AZM and an extended $t_{1/2} > 200$ hours was found.^{2,3,11,12}

In CF, PMNNs accumulate in the airways and contribute to the destruction of airway tissue by the

Received for publication June 17, 2005; accepted October 27, 2005.

From the *The Hague Central Hospital Pharmacy, Escamplaan 900, The Hague, The Netherlands; and †Department of Pulmonology and Adult Cystic Fibrosis Centre, Haga Hospital Leyenburg, Leyweg 275, The Hague, The Netherlands.

Supported in part by an unrestricted educational grant from Pfizer, Capelle a/d IJssel, The Netherlands.

Reprints: E. B. Wilms, The Hague Central Hospital Pharmacy, Escamplaan 900, The Hague, The Netherlands (e-mail: e.wilms@ahz.nl).

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release of proteases.^{8,15} CF patients suffer from a chronic pulmonary infection, which results in an elevated PMNN count and accumulation of PMNNs in airway tissue. This might influence AZM kinetics and distribution in patients with CF.

Therefore, we decided to determine AZM in plasma, isolated PMNNs, and whole blood during a sampling period long enough to detect a terminal $t_{1/2}$ of approximately 200 hours. In addition, we determined the concentration of AZM in sputum samples.

The main objective of this study was to describe the pharmacokinetics of AZM and blood-cell distribution during a 10-day period after chronic use of AZM, 500 mg once daily, in patients with CF. Furthermore, we sought to calculate the degree of enrichment in PMNNs during chronic use of AZM and to evaluate the relationship between AZM concentrations in plasma, blood, PMNN, and sputum.

PATIENTS AND METHODS

Adult patients with CF were recruited from the Leyenburg Hospital Adult Cystic Fibrosis Center. Patients who had received AZM 500 mg once daily for more than 35 days and had chronic *P. aeruginosa* infection (confirmed with at least 2 positive cultures in the last 6 months) were included in the study. The study protocol was approved by the institutional review board, and patients gave written, informed consent before study participation.

Drug Administration and Sampling

AZM was administered 500 mg once daily during at least 35 days (Zithromax[®] tablets 500 mg, Pfizer, Capelle a/d IJssel, The Netherlands) before the 10-day sampling period. AZM therapy was ceased at the end of the first day of the 10-day sampling period and restarted after completion of the study on day 10.

Venous lithium-heparinized blood (119 IU li-heparin/7 mL tube, VacutainerTM Becton-Dickinson, Alphen a/d Rijn, The Netherlands) samples were collected at each time point. On day 1, the samples were collected before administration and 1, 2, 3, 4, 6, and 8 hours after the last dose of AZM. A single sample also was collected between days 4 and 7 and on day 10.

All expectorated sputum was collected during 24 hours on the day before initial blood sample collection, before the sample drawn at days 4 to 7, and before day 10.

Isolation Procedure of Polymorphonuclear Neutrophils and Bioanalysis of AZM

PMNNs were isolated from lithium-heparinized venous blood samples. A differential blood cell count was made. To 6 mL of blood, 6 mL of phosphate-buffered saline (PBS, pH 7.4, Mallinckrodt-Baker, Deventer, The Netherlands) was added. The diluted blood-PBS mixture was transferred into a separation tube with a 6-mL layer of Ficoll-Paque Plus density separation medium

(Amersham Biosciences, Uppsala, Sweden) and centrifuged for 15 minutes (1250 G) at 21°C. The supernatant and Ficoll-Paque layer were removed and the cell pellet with neutrophils and erythrocytes was incubated during 15 minutes with 45 mL of NaCl 0.2% at 2°C to 6°C to lyse the erythrocytes. PMNNs were isolated by centrifugation (5 minutes 465 G) and resuspended in 6 mL PBS. A differential cell count was performed to determine the number of isolated neutrophils. After centrifugation (5 minutes 465 G) the supernatant was removed and the cell pellet was kept at -30°C until determination of AZM.

Detailed specifications and validation of the methodology of cell isolation and AZM quantification in blood, plasma, and PMNNs are described elsewhere.¹⁶ A brief description of the method and the key performance parameters follows: A high-performance liquid chromatographic method with pre-column derivatization and fluorescence detection was used for the quantification of AZM in blood, plasma, isolated PMNNs, and sputum. Clarithromycin (CLM, Abbott, Queenborough, UK) was used as an internal standard and AZM dihydrate salt (Pfizer Inc., New York) was used as reference. Pre-column derivatization was performed using 9-fluorenylmethyloxycarbonyl-chloride (Sigma-Aldrich, Zwijndrecht, The Netherlands). Analytical separation was performed by using a C18 column as stationary phase and a mixture of 760 mL acetonitrile and 240 mL 0.02 M phosphate buffer (0.65 g potassium dihydrogen phosphate in 240 mL water adjusted to pH 7.7 with potassium hydroxide 10%) as mobile phase. Fluorometric detection of the analytes was used for quantification (λ -excitation 267 nm and λ -emission 317 nm).

Calibration curves in plasma and blood (0–1.5 mg/L) were used for determination in plasma and blood. A calibration curve in water (0–9 mg/L) was used for determination in sputum and the PMNN fraction.

Plasma, blood, PMNNs, and sputum samples were kept at -30°C until determination. Quantification of AZM in PMNN samples was performed after thawing and the addition of distilled water to a total volume of 1.2 mL. AZM was quantified in duplicate in 0.5-mL aliquots in plasma, blood, and PMNNs.

The lower limit of quantitation of AZM was 0.042 ± 0.017 mg/L in plasma, 0.119 ± 0.065 mg/L in blood, and 0.072 ± 0.036 mg/L in water. Linearity was assessed from 0 to 1.5 mg/L in plasma and blood and from 0 to 9 mg/L in water.

Recovery and intra-assay variation (expressed as %CV) from plasma at 0.635 mg/L AZM was 103% ($n = 6$, %CV = 1.5%) and of 1 mg/L CLM was 100% ($n = 6$, %CV = 0.8%). The recovery from blood of 0.635 mg/L AZM was 94.5% ($n = 6$, %CV = 2.3%) and of 1 mg/L CLM was 99.4% ($n = 6$, %CV = 1.6%). Inter-assay variation at 0.635 mg/L ($n = 6$) and 1.182 mg/L ($n = 5$) in plasma were 2.2% and 2.8%, respectively. In blood the inter-assay variation at 0.295 mg/L ($n = 6$) and 0.739 mg/L ($n = 6$) were 2.4% and 3.9%, respectively.

Intra-assay variation of the combined PMNN separation procedure and AZM quantification was 4% ($n = 4$) at 1.35 mg/L and 14% ($n = 4$) at 0.61 mg/L.

Validation of the Bioanalysis of AZM in Sputum

Quantification of AZM in sputum was performed after thawing and vortex mixing of the sputum samples using glass pearls. No liquefying agents were used. For each sputum sample, 2 volumes (50 and 250 μ L) were assayed in duplicate. After addition of 200 μ L of a saturated di-sodium carbonate (0.4 mg/mL in water, with a pH of approximately 11) an extraction with diethylether (both from Merck, Darmstadt, Germany) was performed. The concentration was calculated for each sample volume producing results within the range of the calibration curve (0–9 mg/L).

To validate the extraction and bioanalysis in sputum, a reference curve prepared in AZM-free sputum from patients with CF was compared with a reference curve in water in a concentration range of 0.94 to 9.4 mg/L. CLA 4.6 μ g per 250- μ L sample was added as internal standard. The recovery, linearity, intra-assay variation, and LLQ were determined.

Pharmacokinetic Data Analysis

The concentration of AZM was determined in plasma, blood, and PMNNs. The amount quantified in the cell pellet was divided by the cell count (in the final washing fluid) and corrected for loss of PMNNs. The amount of AZM in PMNNs has been expressed as the amount of AZM in PMNNs in 1 L of blood. Also the amount of AZM per PMNN was calculated and subsequently divided by the mean cell volume of human PMNN (334 femtoliter),¹⁷ providing an estimation of the concentration of AZM in the PMNN.

Pharmacokinetic data analyses were performed with MW/Pharm. (version 3.50, Medware, Groningen, The Netherlands).¹⁸ Plasma, blood, and PMNN concentration data points were weighted against the variation of the calibration curve.

RESULTS

Eight patients with CF (5 males; mean age, 29.6 (range, 22–40) years; mean weight, 67 (range, 57–75) kg)

were included in the study. All patients met the inclusion criteria and had received AZM 500 mg once daily for more than 35 days before inclusion. Concomitant medication for CF included oral omeprazole ($n = 5$), prednisone ($n = 5$), pancreatic enzymes ($n = 5$), vitamins A, D, E, and K ($n = 6$), ursodeoxycholic acid ($n = 3$), ibuprofen ($n = 2$), calcium carbonate ($n = 1$), nystatin ($n = 1$), intravenous tobramycin ($n = 2$), meropenem ($n = 3$), ceftazidime ($n = 1$), piperacillin/tazobactam ($n = 4$), colistin ($n = 6$), and the following inhaled therapies: salbutamol ($n = 7$), tobramycin ($n = 2$), hypertonic saline ($n = 4$), dornase- α ($n = 5$), colistin ($n = 5$), and corticosteroids ($n = 8$).

Validation of the Bioanalysis of AZM in Sputum

Comparing the reference curve in water and in cystic fibrosis sputum, a correlation coefficient of 0.999 was determined (concentration range, 0.94–9.4 mg/L). A blank sample fell within the 95% confidence interval of the calibration curve. LOQ was 0.03 mg/L in water and 0.33 mg/L in sputum. Recovery from sputum spiked with 4.68 mg/L AZM was 95% ($n = 8$, %CV = 3.1%). No interferences with the assay were seen in AZM-free sputum from patients with CF.

Pharmacokinetics of AZM

AZM kinetics were best described with a 2-compartment model. This was characterized by a short distribution half-life in plasma (mean and standard deviation: 0.1 hours $\pm$ 0.02 hours) and an extended elimination half-life ($t_{1/2\beta}$) in plasma (102 hours $\pm$ 20 hours), blood (180 hours $\pm$ 68 hours), and in PMNNs (289 hours $\pm$ 166 hours).

The key pharmacokinetic data are summarized in Table 1. The combined patient concentration – time curves of AZM in plasma, blood, and PMNNs (mean $\pm$ standard deviation) are depicted in Figure 1.

The correlation between AZM concentrations in all blood and in all PMNNs samples was 0.731. Correlation between AZM concentrations in blood samples and PMNN samples from $t = 8$ hours until $t = 220$ hours was 0.94. During this interval, an average of 73% of the AZM present in blood could be found in PMNNs (Fig. 2).

In 5 patients, sputum samples were obtained at 3 time points: 0 to 24 hours before, and 96 to 120 hours and

TABLE 1. Key Mean Pharmacokinetic Data of AZM in Plasma, Blood, and PMNNs After >35 Days of 500-mg Once Daily in CF Patients ($n = 8$)

AZM	Plasma (mg/L)	PMNN (mg/L)	Blood (mg/L)	PMNN (%)	Plasma (%)
C_{max}	0.67 $\pm$ 0.31	1.44 $\pm$ 0.69	2.01 $\pm$ 0.74	72	33
t_{max} (hr)	3 $\pm$ 1.6	4 $\pm$ 0.4	3 $\pm$ 1.1		
C_{min} $t = 0$ (hr)	0.11 $\pm$ 0.05	0.55 $\pm$ 0.32	0.86 $\pm$ 0.43	64	13
C_{min} $t = 220$ (hr)	0.04 $\pm$ 0.02	0.3 $\pm$ 0.14	0.37 $\pm$ 0.2	81	11
$t_{1/2\alpha}$ (hr)	0.1 $\pm$ 0.02	—	2.9 $\pm$ 1		
$t_{1/2\beta}$ (hr)	102 $\pm$ 20	289 $\pm$ 166	178 $\pm$ 68		
AUC _{0–24 hr} (mg·hr/L)	5.3 $\pm$ 1.4	18.5 $\pm$ 8.9	27.8 $\pm$ 10.6		

Data are means $\pm$ standard deviations or percentages.

Concentration in PMNNs is expressed as the amount of AZM detected in isolated PMNNs per 1 L of blood.

FIGURE 1. AZM time concentration profiles during a 10-day sampling period after long-term (>35 days) use of AZM 500 mg per day. Average value ($n=8$, $\pm$ SD) of AZM in plasma (cross, upper part), in PMNNs (square, middle part), and in blood (triangle, bottom part) are presented. Concentration in PMNNs was expressed as amount quantified in isolated PMNNs per liter blood.

196 to 220 hours after the last dose. In 2 patients, sputum samples were obtained at 2 time points, and 1 patient could only produce sufficient sputum during 1 sampling period. Concentrations of AZM in sputum up to 53 mg/L were found (Table 2).

The calculated intracellular concentration in PMNNs and the PMNN to plasma ratio is summarized in Table 2. The maximum intracellular concentration calculated (mean of combined patient data at $t=8$ hours) was 305 ± 82 mg/L. The PMNN to plasma ratio was not

FIGURE 2. Correlation between AZM in blood (x-as) versus AZM in PMNNs. The combined data from 8 patients receiving AZM 500 mg per day chronically (>35 days) were used. The data from $t=8$ hours until $t=220$ hours after the last dose of AZM were used to calculate a correlation coefficient of 0.94 and a fraction of 0.73 (73%) of AZM in blood incorporated in PMNNs.

constant, as is depicted in the PMNN to plasma ratio versus time curve in Figure 3.

For sputum the correlation between pharmacokinetic parameters and concentration of AZM in the first sample (0–24 hours) was calculated. There was no correlation between the concentration of AZM in sputum and the following parameters: area under the curve (AUC) 0 to 24 hours, maximum AZM concentrations and predose AZM concentrations in plasma, blood, and PMNNs. There was a weak correlation between $t_{1/2\beta}$ in blood and sputum concentrations between 0 to 24 hours ($r=0.89$).

DISCUSSION

The pharmacokinetics of AZM in blood are primarily determined by the accumulation and prolonged retention of AZM in PMNNs. The elimination half-lives in plasma, blood, and PMNNs were comparable to the data found by Wildfeuer et al³ after short-term (3 days) administration in healthy subjects, as was the C_{max} in plasma (0.67 ± 0.31 mg/L vs. 0.64 ± 0.27 mg/L). The mean C_{max} in blood was substantially increased to 2 ± 0.7 mg/L after chronic use versus ± 1 mg/L after 3 days of AZM therapy.

We found a mean concentration of 305 ± 82 mg/L in PMNNs at $t=8$ hours. This value was substantially higher compared with the 119 ± 31 mg/L that Wildfeuer found at $t=6$ hours after 3 days dosing of 500 mg per day in healthy volunteers. Comparing these results, we have to be aware that the methodology was different. Wildfeuer calculated the intracellular concentration after determination of the intracellular water space. We used an average volume of the PMNN. Taking the long half-life of AZM in PMNNs into account, we can assume a steady-state situation had not been established after 3 days of dosing

TABLE 2. AZM PMNN to Plasma Ratio and Concentration in Sputum

	AZM in Plasma (mg/L) (n = 8)	AZM in PMNNs (mg/L) (n = 8)	PMNN to Plasma Concentration Ratio (%)	AZM in Sputum (mg/L)
C _{max} plasma	0.67 ± 0.31	300 ± 125	448	—
C _{min} t = 0 hr	0.11 ± 0.05	231 ± 71	2100	32 ± 16 (n = 7)
C t = 96 to 120 hr	—	—	—	17 ± 9 (n = 6)
C _{min} t = 220 hr	0.04 ± 0.02	118 ± 41	2950	20 ± 11 (n = 7)

Data are means ± standard deviations or numbers.

The concentration in PMNNs is calculated from the amount per PMNN divided by the mean cell volume of human PMNN (334 femtoliter). The concentration of AZM in sputum was determined in a sample from a 24-hour collection of spontaneously produced sputum.

AZM, which may explain our higher intracellular concentration.

The concentration ratio between AZM in PMNN and plasma in our study was between 450 and 2950 to 1. Mandell and Coleman¹⁹ found a concentration ratio of 517 in an in vitro experiment and Wildfeuer found a concentration ratio between PMNNs and plasma of 177 at 3 hours and 1814 at 120 hours after administration of 500 mg per day for 3 days.³ The decline and rise of the concentration ratio is more or less reciprocal to the plasma concentration of AZM and not a constant factor, not even at steady state. The V-shaped pattern of the concentration ratio versus time is predominantly determined by the variation in plasma concentrations during the absorption and distribution phase and describes the lag-time between resorption in plasma and uptake in PMNNs. Whether this difference is the result of saturation of transport or just the time needed to establish a new equilibrium between internal and external concentration remains unknown. Other explanations, such as an increased loss at higher intracellular concentrations during the process of PMNN separation, cannot be excluded.

FIGURE 3. PMNN/plasma ratio versus time curve (mean ± SD). The PMNN/plasma ratio was calculated from the concentration in PMNNs and the concentration in plasma. The concentration in PMNNs was calculated from the amount per PMNN divided by the mean cell volume of human PMNN (334 femtoliter).

During the absorption and distribution phase, there was no correlation between the concentration in blood and in PMNNs. However, for samples drawn 8 hours and later (after the absorption and distribution phase), the correlation coefficient was 0.94 and, on average, 73% of AZM present in blood could be retrieved from PMNNs. This correlation is sufficient to relate PMNN concentration directly to whole blood concentration of AZM.

Leakage of AZM from PMNNs during the separation procedure could have influenced our results. In this study we used an analytical assay and separation method for PMNNs, the details of which have been described previously.¹⁶ Using this method we were not able to demonstrate leakage of AZM from PMNNs during the isolation process. From the amount measured in isolated PMNNs, after correction for incomplete PMNN isolation, we found on average 87% of the AZM in the PMNNs and plasma fraction (compared with blood, calculated from all patient samples). Part of the difference between blood concentration and the sum of plasma and PMNN concentration could be explained by the accumulation of AZM in other lysosome containing blood cells, such as monocytes. Therefore, leakage from PMNNs in the isolation process may have been less than the difference between 87% and 100% and did not influence our findings.

At C_{max}, the sum of the concentration in plasma and in PMNNs (average of combined patient data) was > 100%. This can only be explained by the variation found in the selection and quantification of PMNNs and the analytical method.

The intracellular concentration in PMNNs was calculated from the amount per PMNN and the mean volume of a PMNN. This would reflect the real concentration if AZM was distributed through the entire PMNN. From the results of Hand and Gladue, we can conclude that the dibasic molecule AZM (pK_a = 8.1 and 8.8) is merely concentrated in the acidic inner environment of intracellular lysosomes.^{20–22} The difference in pH is the most probable driving force toward accumulation in intracellular lysosomes.^{20–24}

Finally, we were interested in the amount of AZM at the site of infection: the lung. Sampling techniques that could be applied were: bronchial alveolar lavage (BAL), sampling spontaneously coughed up sputum, and

sampling sputum after stimulation of secretion. The advantage of a BAL sample is its origin from the alveolar space. The disadvantage of a BAL sample is its unknown dilution factor and the fact that BAL is an unpleasant and distressing procedure for our group of patients. Sputum is more easily available and is formed not only in the alveolar space but also throughout the entire lung. We assumed a preferable relationship between sputum and the concentration in the lung without stimulation of sputum secretion and sampled sputum, which was produced by spontaneous coughing during a 24-hour period.

In the sputum of patients with CF, profound retention of neutrophils is found.²⁵ The elevated concentration of AZM in sputum can, in our opinion, be explained when we see PMNNs as the transporting vehicle for AZM toward the lung. From our results, we cannot conclude whether AZM in sputum is associated with PMNNs, bound to other sputum constituents, or is freely soluble.

This begs the question as to what sputum AZM concentration is of clinical significance in the treatment of a chronic infection with *P. aeruginosa*. Tetteda et al¹⁰ found inhibition of quorum sensing factor production at concentrations of 2 mg/L in the *P. aeruginosa* strain PAO1. Takeoka et al⁹ found a MIC of 100 mg/L toward both mucoid and non-mucoid strains of *P. aeruginosa*, and his findings suggest an increase of *P. aeruginosa* phagocytosis by PMNNs after exposure to sub MIC concentrations of AZM. Tateda et al¹⁰ found a reduction of the viability of *P. aeruginosa* after exposure for 48 hours to AZM at a concentration of 0.5 mg/L.

To be able to relate the concentrations we found in sputum (20–54 mg/L) to the concentrations at which AZM has been shown to influence *P. aeruginosa* growth during in vitro experiments, more information about the way in which AZM is incorporated in sputum and its ability to penetrate *P. aeruginosa* biofilm should be gained. Our results clearly demonstrate that sputum is a reservoir with a relevant elevated AZM content.

We found no correlation between AUC of AZM in plasma, blood, and PMNNs, and the concentration of AZM in sputum in the sample collected at $t = 0$ until $t = 24$ hours. Absence of a correlation between plasma and sputum concentrations may indicate that transport from blood to sputum is not described by a linear relationship as is the case in passive diffusion. According to our hypothesis, that AZM is transported to the lung through PMNNs, other variables may influence sputum concentrations: the number of PMNNs in blood, the number of PMNNs migrating into the lung, the amount of sputum produced, and the amount of sputum excreted will all have influence on the concentration of AZM in sputum.

We conclude that chronic administration of AZM leads to higher accumulation in PMNNs compared with short-term use. Furthermore, we conclude that sputum concentrations are elevated far above concentrations found in plasma and blood. We suggest that a less

frequent (for instance, once weekly) dosing regimen be compared with daily dosing regimens to assess clinical efficacy, pharmacokinetics, and accumulation in sputum of AZM.

ACKNOWLEDGMENTS

The authors thank H. Gongrijp, H. Trumple, and N. Boone, who helped with the separation of polymorphonuclear neutrophils and bioanalysis of AZM, and Dr. S. Bell for assistance.

REFERENCES

1. Mazzei T, Mini E, Novelli A, et al. Chemistry and mode of action of macrolides. *J Antimicrob Chemother.* 1993;31(Suppl C):1–9.
2. Amsden GW, Gray CL. Serum and WBC pharmacokinetics of 1500 mg of azithromycin when given either as a single dose or over a 3 day period in healthy volunteers. *J Antimicrob Chemother.* 2001;47:61–66.
3. Wildfeuer A, Laufen H, Zimmermann T. Distribution of orally administered azithromycin in various blood compartments. *Int J Clin Pharmacol Ther.* 1994;32:356–360.
4. Equi A, Balfour-Lynn IM, Bush A, et al. Long-term azithromycin in children with cystic fibrosis: a randomised, placebo-controlled crossover trial. *Lancet.* 2002;360:978–984.
5. Jaffe A, Francis J, Rosenthal M, et al. Long-term azithromycin may improve lung function in children with cystic fibrosis. *Lancet.* 1998;351:420.
6. Saiman L, Marshall BC, Mayer-Hamblett N, et al. Macrolide Study Group. Azithromycin in patients with cystic fibrosis chronically infected with *Pseudomonas aeruginosa*: a randomized controlled trial. *JAMA.* 2003;290:1749–1756.
7. Wolter J, Seeney S, Bell S, et al. Effect of long-term treatment with azithromycin on disease parameters in cystic fibrosis: a randomised trial. *Thorax.* 2002;57:212–216.
8. Ichimiya T, Takeoka K, Hiramatsu K, et al. The influence of azithromycin on the biofilm formation of *Pseudomonas aeruginosa* in vitro. *Chemotherapy.* 1996;42:186–191.
9. Takeoka K, Ichimiya T, Yamasaki T, et al. The in vitro effect of macrolides on the interaction of human polymorphonuclear leukocytes with *Pseudomonas aeruginosa* in biofilm. *Chemotherapy.* 1998;44:190–197.
10. Tateda K, Comte R, Pechere JC, et al. Azithromycin inhibits quorum sensing in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother.* 2001;45:1930–1933.
11. Conte JE Jr, Golden J, Duncan S, et al. Single-dose intrapulmonary pharmacokinetics of azithromycin, clarithromycin, ciprofloxacin, and cefuroxime in volunteer subjects. *Antimicrob Agents Chemother.* 1996;40:1617–1622.
12. Morris DL, De Souza A, Jones JA, et al. High and prolonged pulmonary tissue concentrations of azithromycin following a single oral dose. *Eur J Clin Microbiol Infect Dis.* 1991;10:859–861.
13. Olsen KM, San Pedro G, Gann LP, et al. Intrapulmonary pharmacokinetics of azithromycin in healthy volunteers given five oral doses. *Antimicrob Agents Chemother.* 1996;40:2582–2585.
14. Patel KB, Xuan D, Tessier PR, et al. Comparison of bronchopulmonary pharmacokinetics of clarithromycin and azithromycin. *Antimicrob Agents Chemother.* 1996;40:2375–2379.
15. Re C. Use of modulators of airway inflammation in patients with CF. *Clin Rev Allergy Immunol.* 2002;23:29–39.
16. Wilms E, Trumple H, Veenendaal W, et al. Quantitative determination of azithromycin in plasma, blood and isolated neutrophils by liquid chromatography using pre-column derivatization with 9-fluorenylmethyloxycarbonyl chloride and fluorescence detection. *J Chromatogr B.* 2005;814:37–42.

17. Nibbering P, Zomerdijk T, Corsel-van Tilburg A, et al. Mean cell volume of human blood leucocytes and resident and activated murine macrophages. *J Immunol Meth.* 1990;129:143–145.
18. Proost JH, Meijer DK. MW/Pharm, an integrated software package for drug dosage regimen calculation and therapeutic drug monitoring. *Comput Biol Med.* 1992;22:155–163.
19. Mandell GL, Coleman E. Uptake, transport, and delivery of antimicrobial agents by human polymorphonuclear neutrophils. *Antimicrob Agents Chemother.* 2001;45:1794–1798.
20. Hand WL, Hand DL. Characteristics and mechanism of azithromycin accumulation and efflux in human polymorphonuclear leukocytes. *Int J Antimicrob Agents.* 2001;18:419–425.
21. Gladue RP, Snider ME. Intracellular accumulation of azithromycin by cultured human fibroblasts. *J Antimicrob Chemother.* 1990;34:1056–1060.
22. Galichet, ed. *Clarke's Analysis of Drugs and Poisons.* 3rd ed. London: Pharmaceutical Press; 2004.
23. De Duve C, De Barse T, Poole B, et al. Lysosomotropic agents. *Biochem Pharmacol.* 1974;23:2495–2531.
24. Hollemans M, Oude Elferink R, De Groot PG, et al. Accumulation of weak bases in relation to intralysosomal pH in cultured human skin fibroblasts. *Biochim Biophys Acta.* 1981;643:140–151.
25. Hubeau C, Lorenzato M, Couetil J, et al. Quantitative analysis of inflammatory cells infiltrating the cystic fibrosis airway mucosa. *Clin Exp Immunol.* 2001;124:69–76.