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Levels of Azithromycin and Alpha-1 Acid Glycoprotein in Serum in Patients with Community-Acquired Pneumonia

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After an oral dose of 500 mg of azithromycin in patients with community-acquired pneumonia, their serum concentrations ranged between 0.06 and 0.25 mg/liter during the first 12 hours; the calculated percentages of unbound drug varied between 45 and 86%. This study shows that in these patients, the total levels of azithromycin in serum are lower than those expected and that the percentage of bound drug is clinically irrelevant.

The pharmacokinetics of azithromycin, one of the new macrolides, are characterized by high tissue concentrations and relatively low serum concentrations. In healthy volunteers, an oral dose of 500 mg of azithromycin leads to maximum serum concentrations of 0.4 to 0.45 mg/liter within 2 to 3 h (10). Azithromycin mainly binds to α_1-acid glycoprotein (AAG) (10). Concentrations of AAG may increase two- to fourfold during an infection (5), which could result in a decrease of the unbound, i.e., active, fraction of the drug (12). However, although the influence of an infection on the pharmacokinetics of azithromycin has not yet been examined in detail, dosage schedules are based on pharmacokinetics in healthy subjects.

The aim of the present study was to establish whether the concentration of azithromycin in patients with community-acquired pneumonia (CAP) corresponds to that found for healthy volunteers. The concentration of AAG was used to calculate the estimated percentage of unbound drug.

The Review Committee of the Leiden University Hospital had no objections to this study. Patients with CAP participated in the present study after giving informed consent. Some of the exclusion criteria were the need for intravenous treatment or the presence of disorders which affect the absorption of orally administered drugs. On the 1st day, the patients received 500 mg of azithromycin orally twice, which was followed by 500 mg once daily for the next 4 days. Eight patients, including four females, ranging in age from 32 to 75 years old were enrolled. *Streptococcus pneumoniae* was detected in four patients (all sensitive to the study drug), and *Legionella pneumophila* and *Mycoplasma pneumoniae* were detected each in one patient. In two patients, no pathogen could be found. Only in one patient (subject 5), who had a mycoplasmal pneumonia, did treatment fail.

Blood samples were collected immediately before and 3 and 12 h after the first and second doses of azithromycin. Total (bound plus unbound) serum concentrations were determined by an agar well diffusion bioassay (3). The amount of AAG was determined by kinetic nephelometry (Beckman Array Protein System; Beckman Instruments, Inc., Galway, Ireland) in blood samples collected prior to the first dose of azithromycin.

Although physical-chemical properties of AAG can change during disease states, values of K_d (dissociation constant) and CB_{max} (maximum binding capacity) are found in a relatively small range (6). Therefore, we used the results of a protein-binding study of azithromycin in healthy volunteers performed by Foulds et al. (2) to estimate K_d and CB_{max} values of AAG for azithromycin, according to the following equation:

$$CB = CB_{max} \times CU/(CU + K_d) \quad (1)$$

where CB and CU are the serum concentrations of bound and unbound drug, respectively. Equation 1 was fitted by nonlinear regression (NONLIN, SYSTAT 5.0; Systat, Inc., Evanston, Ill.) allowing separate estimates of the respective constants, which yielded a K_d of 0.11 mg/liter and a CB_{max} 0.071 mg/liter. The algorithm used a quasi-Newton nonlinear least-squares estimator. From these results, the binding capacity for azithromycin per gram of AAG was calculated by dividing 0.071 mg/liter (CB_{max}) by 0.9 g/liter, i.e. the mean of the values for healthy volunteers (5). The values of K_d and CB_{max} were then used to estimate the actual binding to AAG in our patients, with the product of the individual AAG concentration and the binding capacity per gram of AAG as an estimate of CB_{max}. With these parameters, the CU values were calculated from the measured values of the total serum concentration (C_{tot}) as follows (1):

$$CU = 0.5 \times (C_{tot} - K_d - CB_{max} + \sqrt{(C_{tot} - K_d - CB_{max})^2 + 4\,K_d \times C_{tot}}) \quad (2)$$

During the first 12 h of treatment, the C_{tot}s of azithromycin varied between 0.06 and 0.25 mg/liter after 3 h (Table 1) and between 0.03 and 0.12 mg/liter after 12 h; 3 h later, the second dose levels ranged from 0.28 to 0.55 mg/liter. In three patients, not all blood samples were obtained. One patient refused venipuncture; in the others, there were logistical problems. In four patients, AAG levels were above normal, i.e., higher than 0.6 to 1.2 mg/liter. Three hours after the first dose, the estimated percentages of unbound azithromycin varied between 45 and 86%, and 3 h after the second dose, they ranged between 62 and 87%.

The results of this study show that in all patients, C_{tot} of azithromycin remained below the expected values during the first 12 h of treatment. In none of the patients did the percentage of bound drug seemed to be clinically relevant. As a result of the low peak levels achieved, the total concentration of azithromycin in the patients was less than that predicted for the first 12 h of treatment. However, it is possible that peak levels for patients are not reached within 2 to 3 h after administration, as in healthy volunteers (10).

S. pneumoniae is a common cause of CAP (11); in the event of bacteremia, the fatality rate is up to 26%, and about half of

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TABLE 1. Concentrations of azithromycin and AAG and percentages of free drug

Subject	Sex	Age (yr)	Wt (kg)	AAG (g/liter)	C_{tot} of azithromycin (mg/liter)			
					After 3 h		After 15 h	
					Total	Free (%)[a]	Total	Free (%)[a]
1	M	69	63	0.90	0.06	0.04 (66)	0.32	0.26 (81)
2	M	57	75	1.01	0.11	0.07 (64)	0.45	0.38 (84)
3	F	38	57	2.13	0.11	0.05 (45)	0.29	0.18 (62)
4	F	32	65	1.00	0.24	0.18 (75)	0.55	0.48 (87)
5	M	60	76	1.82	0.19	0.11 (58)		
6	M	68	68	1.43	0.25	0.17 (68)	0.28	0.20 (71)
7	F	75	59	1.85	0.14	0.08 (57)		
8	F	37	100	0.61	0.22	0.19 (86)		

[a] See Materials and Methods for description of these calculations.

these patients die within 24 h of admission (7, 9). The MIC of azithromycin for *S. pneumoniae* is 0.27 mg/liter (10), a level reached in none of the patients during the first 12 h of treatment. Nevertheless, azithromycin has been shown to be a proper treatment for CAP, including some patients with pneumococcal bacteremia (8, 10). However, some of the exclusion criteria used, e.g., the need for intravenous therapy for patients older than 65 years of age may have biased the study results. The efficacy of the drug has been explained by its high levels in tissue and accumulation in granulocytes and macrophages, resulting in target delivery (10). The serum concentrations do not seem to be good predictors of clinical efficacy, although experimental results indicate that serum concentrations, at least of erythromycin, are indeed of predictive value (4). The question of whether azithromycin with a dosage regimen used in this study results in an optimal treatment for bacteremic pneumococcal pneumonias remains.

We conclude that a dosage schedule based on healthy volunteers that is used for patients with CAP does not provide the expected serum concentrations during the first 12 h of treatment. Therefore, more pharmacokinetical studies in patients have to be performed to assess the best dosage regimen.

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