Azithromycin Dose To Maximize Efficacy and Suppress Acquired Drug Resistance in Pulmonary *Mycobacterium avium* Disease

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*Mycobacterium avium* complex is now the leading mycobacterial cause of chronic pneumonia in the United States. Macrolides and ethambutol form the backbone of the regimen used in the treatment of pulmonary disease. However, therapy outcomes remain poor, with microbial cure rates of 4% in cavitary disease. The treatment dose of azithromycin has mostly been borrowed from that used to treat other bacterial pneumonias; there are no formal dose-response studies in pulmonary *M. avium* disease and the optimal dose is unclear. We utilized population pharmacokinetics and pharmacokinetics/pharmacodynamics-derived azithromycin exposures associated with optimal microbial kill or resistance suppression to perform 10,000 patient Monte Carlo simulations of dose effect studies for daily azithromycin doses of 0.5 to 10 g. The currently recommended dose of 500 mg per day achieved the target exposures in 0% of patients. Exposures associated with optimal kill and resistance suppression were achieved in 87 and 54% of patients, respectively, only by the very high dose of 8 g per day. The azithromycin susceptibility breakpoint above which patients failed therapy on the very high doses of 8 g per day was an MIC of 16 mg/liter, suggesting a critical concentration of 32 mg/liter, which is 8-fold lower than the currently used susceptibility breakpoint of 256 mg/liter. If the standard dose of 500 mg a day were used, then the critical concentration would fall to 2 mg/liter, 128-fold lower than 256 mg/liter. The misclassification of resistant isolates as susceptible could explain the high failure rates of current doses.

In the United States, pulmonary *Mycobacterium avium* complex (MAC) disease is more common than tuberculosis (1). While there has been a decline in the rate of tuberculosis in the United States since 1992, with a rate of 3.0 per 100,000 in 2013, the prevalence of MAC has been increasing (2, 3). The minimum estimate of 2-year period prevalence of pulmonary nontuberculous mycobacteria from a clinical study of Oregon residents between 2005 and 2006 was 8.6 per 100,000 in the general population: MAC was responsible for 88% of the cases (4). Although it is universally acknowledged that there is an urgent need to develop new antituberculosis agents to add to the half-dozen new drugs that have become available, the pipeline for new anti-MAC drugs is totally empty. The two important drugs for the treatment of pulmonary MAC are macrolides and ethambutol, but in practice patients also receive rifampin and aminoglycosides (1, 5). The two macrolides used in such regimens are either clarithromycin or azithromycin. Therapy duration is for several years. However, therapy outcomes with this combination regimen are very poor, with only 50 to 60% response, and the response rates were from noncomparative single-center studies of small numbers of patients, as well as no intention to treat analyses (1, 5). When strict microbiologic criteria such as culture conversion were used to define cure in a prospective study of 91 patients from 17 U.S. cities, the cure rate was only 4% in cavitary disease and 24% in noncavitary pulmonary disease (6). Indeed, the guidelines propose that for some patients with cavitary pulmonary MAC, the disease should be viewed as incurable (1). Recommended azithromycin doses vary from 500 to 600 mg three times a week or 250 mg per day, but these are not based on a formal dose-response clinical study or even formal pharmacokinetic/pharmacodynamic (PK/PD) work (1).

In PK/PD studies in the hollow-fiber system model of MAC (HFS-MAC), the maximal kill by ethambutol was limited, with monotherapy at maximal doses achieving bacterial burden higher than at the start of treatment (7). Indeed, it is believed that most microbial kill achieved by the regimen is derived from macrolides, so that when there is resistance to the macrolide, therapy fails (8, 9). However, the optimal doses for the treatment of pulmonary MAC are unclear but were derived mostly from the azithromycin dose that the manufacturers took to market for more mundane bacterial pneumonias. In the absence of clinical trials, laboratory-derived PK/PD exposures of antibacterial agents have been used to perform dose-response studies in silico for many types of infections. In the case of agents used to treat tuberculosis (TB), for example, such an approach has been found to have a forecasting accuracy of 94% of doses and exposures later identified as optimal in combination therapy in the clinic (10–12). We were interested in using this approach to identify the azithromycin dose that would achieve two important therapeutic outcomes: (i) optimize microbial kill of intracellular pulmonary MAC and (ii) suppress acquired drug resistance. Experience with TB monotherapy studies has demonstrated that the optimal antibiotic exposures or concentrations identified in monotherapy in preclinical models such as the hollow-fiber system are the same as in combination therapy in patients (10–12). In other words, each drug component in the...
combination therapy should be optimized as monotherapy if it is to be expected to perform optimally in combination therapy in patients.

Clinical studies to identify optimal azithromycin exposures are complicated by the fact that the drug concentrations measured in the plasma of patients are different from those achieved in lung white cells such as macrophages, the site of MAC infection in lung disease (13, 14). In the HFS-MAC, we identified the accumulation of azithromycin in MAC-infected macrophages with an intracellular-to-extracellular area under the concentration-time curve from 0 to 24 h (AUC₀–2₄) of 8,193-fold (15). Indeed, very similar AUC₀–2₄ penetration ratios were identified in the bronchopulmonary system and alveolar macrophages a decade earlier by Rodvold et al., who demonstrated penetration ratios of 867 to 12,158 in patients (16). By applying mass balance of conservation of mass, the amount of drug in the body at site of infection can thus be inferred from the plasma. This means that optimal exposures in infected lungs can be calculated, when plasma concentrations are known, by taking into account the penetration ratio of the AUCs. The ratios of intracellular (lung macrophage) to plasma concentrations are the same even when the oral extended-release azithromycin (azithromycin-ER) is administered; this formulation may be more useful given the long duration of therapy for pulmonary MAC disease (17). The HFS-MAC has identified the “plasma” (or extracellular) AUC₀–₂₄/MIC ratios associated with 80% of maximal kill (EC₅₀) of intracellular MAC as 3.43, while that associated with suppression of ADR was 7.51 (15). Interestingly, when Muto et al. examined 559 patients with pneumonia caused by various mundane Gram-negative and Gram-positive bacteria, they found the bacteriological and clinical success rates were 95.8 and 100% in patients with an AUC/MIC of >5 compared to 60.0 and 83.3% in those with an AUC₀–₂₄/MIC of <5 (18). Similarly, the plasma AUC/MIC ratio associated with the optimal efficacy of another macrolide, telithromycin, was 3.34 (19). Thus, the AUC/MIC associated with optimal outcome derived in our HFS-MAC are in the same range as those identified in the clinic in patients with pneumonia from other bacteria. We used these findings to examine the probability that different azithromycin-ER doses could achieve, or exceed, the steady-state AUC₀–₂₄/MIC of 3.43 or 7.51 in patients with pulmonary MAC disease. This in silico dose-ranging study was also used to identify the azithromycin susceptibility breakpoint above which patients would be expected to fail therapy.

**Materials and Methods**

**Philosophical underpinnings of study.** Based on PK/PD principles, the relationship between microbial effect and concentration is a deterministic system described by a variety of equations of drug exposure versus bacterial burden size (10). These deterministic relationships are independent of biological system; these relationships are what is translated from a preclinical model system to the clinic. However, the human pharmacokinetic system is a nondeterministic system and is characterized by extensive between-patient and between-occasion pharmacokinetic variability due to identifiable and nonidentifiable reasons. Thus, when patients receive an identical dose, they do not attain the same concentration-time profiles. This variability must be accounted for when designing optimal doses. Moreover, since drug exposure (e.g., AUC/MIC) is a quotient of drug concentration and MIC, the bacterial drug MIC variability must also be accounted for in identification of optimal doses. The MIC is in fact known to have a direct effect in determining microbial response in mycobacterial pulmonary diseases, including MAC (20–22). This also means that there exists an MIC above which there will be little to no microbial kill in patients receiving the maximum tolerated dose (23). That MIC is what identifies the susceptibility breakpoint for the drug against the specific bacterial species.

**Software and hardware.** All simulations were performed on an iMac desktop with a 2.7 GHz Intel Core i5 processor. Monte Carlo experiments were implemented using subroutine PRIOR of ADAPT 5. The Fortran model files were edited to reflect azithromycin pharmacokinetic parameter estimates and variability. Output in .csv files was converted to Microsoft Excel spreadsheets and then exported to GraphPad Prism 6 software for graphing and statistical analysis.

**Azithromycin exposure targets.** The azithromycin exposures to be achieved by the different doses were a plasma steady-state AUC₀–₂₄/MIC of 3.43 for the selection of a susceptibility breakpoint and 7.51 for dose identification. These exposure targets were derived in a prior HFS-MAC study (15). Doses would be expected to kill optimally and suppress development of acquired resistance if they achieved the exposure that suppresses drug resistance, since attaining a target AUC₀–₂₄/MIC of 7.51 also attains the 3.43 target for optimal microbial kill.

**Population pharmacokinetic model parameters.** We utilized the population pharmacokinetic parameter estimates, and the covariance matrix, identified by Muto et al. in 559 patients treated with extended release azithromycin for pneumonia (18). This study was chosen because it is the largest that identified compartmental pharmacokinetic parameters for treatment of pneumonia and included pharmacokinetic sampling at steady state. The pharmacokinetic parameters entered into subroutine PRIOR are shown in Table 1. In the study by Muto et al. there were no estimates for the intercompartmental clearance and peripheral volume interindividual variability. We assigned a variability of 40% for these parameters, which was well within the range observed for clearance and central compartment volume in the same study.

**Azithromycin MIC distribution.** Since clarithromycin MIC testing is used as a surrogate for azithromycin susceptibility, there are no large databases of azithromycin MICs for pulmonary MAC isolates. The best distribution is obtainable from a small study by Griffiths et al., who published the azithromycin MICs from 23 patient isolates as part of a combination therapy study (5). In that study, the MIC₅₀ was 8 mg/liter, and

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**Table 1. Pharmacokinetic parameters entered into subroutine PRIOR versus 10,000-subject simulation output**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Domain of input</th>
<th>Simulation results*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total clearance (liters/h)</td>
<td>Mean 103.0</td>
<td>Median 102.7</td>
</tr>
<tr>
<td></td>
<td>IIV (%) 34</td>
<td>Range 82.74–126.10</td>
</tr>
<tr>
<td>Vol of central compartment (liters)</td>
<td>1,820</td>
<td>1,820</td>
</tr>
<tr>
<td>Absorption constant (h⁻¹)</td>
<td>0.725</td>
<td>0.454</td>
</tr>
<tr>
<td>Intercompartmental clearance (liters/h)</td>
<td>138.0</td>
<td>137.6</td>
</tr>
<tr>
<td>Vol of peripheral compartment (liters)</td>
<td>4,340</td>
<td>4,341</td>
</tr>
</tbody>
</table>

* Values are expressed as medians and ranges because the results had a P value of <0.05 for the D’Agostino and Pearson omnibus normality test and were thus not normally distributed.

b, interindividual variability.
MIC\textsubscript{90} was 32 mg/liter. The MIC distribution is consistent with other studies in which the MAC isolates were of unclear origin that showed an MIC\textsubscript{50} of 16 mg/liter and an MIC\textsubscript{90} of 32 mg/liter in 28 isolates, and another study with blood culture isolates that showed an MIC\textsubscript{50} and an MIC\textsubscript{90} of 32 mg/liter (24, 25).

Monte Carlo experiments. Monte Carlo experiments derive from the work of Ulam and Metropolis in validation of the ENIAC in the Manhattan project (12, 26). These experiments have been used to identify optimal doses for treatment of MAC in the past (7, 27). Here, we specifically implemented these methods, taking into account the intracellular nature of MAC and expected intracellular pulmonary penetration of azithromycin. The implementation was performed in subroutine PRIOR of ADAPT 5. Azithromycin population pharmacokinetic parameter estimates and variability as described by Muto et al. (see above) were the domain of input (18). We then identified a distribution of steady-state AUC\textsubscript{0–24} values achieved in 10,000 patients treated with azithromycin-ER tablets for each of the total daily doses of 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, and 10 g. Target attainment probability was the proportion of 10,000 simulated patients who would achieve a plasma steady-state AUC\textsubscript{0–24}/MIC of 3.43 or 7.51. The target attainment probability was calculated for each dose at each azithromycin MICs of 1, 2, 4, 8, 16, 32, 64, and 128 mg/liter. The cumulative fraction of response (CFR) was then calculated to take into account the MIC distribution, based on the formula:

$$CFR = \sum_{i=1}^{n} PTA_i \cdot F_i$$

where PTA is the probability target attainment at each MIC, and \( F \) is the proportion of isolates at each MIC.

RESULTS

Table 1 compares the pharmacokinetic parameters entered as prior data in ADAPT to those in the 10,000 subject Monte Carlo experiments output. The table shows very good recapitulation of Muto et al. reported pharmacokinetic parameter estimates by the simulations. We used a separate pulmonary MAC study for external validation of the simulations. van Ingen et al. reported pharmacokinetic results in 240 American patients with pulmonary MAC who were treated with 500 mg of azithromycin (28). They identified a 0 to 7 h AUC (AUC\textsubscript{0–7}) of 1.68 ± 0.95 mg·h/liter in these patients. In our simulations, the azithromycin AUC\textsubscript{0–7} in 10,000 simulated subjects treated with 500 mg a day was 1.51 ± 0.09 mg·h/liter. Thus, the AUCs in our simulated subjected was similar to those observed in American patients with pulmonary MAC, justifying our assumptions.

Figure 1A shows the performance of the standard dose of azithromycin MIC\textsubscript{90} was 32 mg/liter.
thromycin of 0.5 g in achieving both the $EC_{80}$ and the $AUC_{0-24}$/MIC that suppresses resistance emergence. Based on this, at no MIC did the standard dose achieve the $AUC_{0-24}$/MIC associated with resistance suppression. For the optimal microbial kill, the target attainment fell to <90% above the MIC of 1.0 mg/liter. Nevertheless, this means that there will be a proportion of patients, especially those with an MIC of <2.0 mg/liter, who will respond to 500 mg a day of azithromycin at optimal kill rates. The overall cumulative fraction of response (CFR) was 0% for both the $EC_{80}$ and resistance suppression. Given that this is the standard dose, we played a “what if” game and examined the role of MICs in achieving the azithromycin exposure associated with the minimal measurable microbial kill, which is at the first inflection of the dose-response curve or the $EC_{20}$ exposure, which was an $AUC_{0-24}$/MIC of 1.29. Figure 1B shows that the standard dose achieves that exposure or exceeds it in isolates with an MIC of up to 2 mg/liter. In other words, there is virtually no microbial kill of MAC starting at concentrations of 4 mg/liter with the standard dose. The CFR for $500 \text{mg}$ achieving the minimum measurable kill was 6.58%. How- ever, regarding resistance suppression exposure, the CFR was still 0%. Figure 2B shows performance of the 2-g dose. The $EC_{80}$ target attainment was ~100% until an MIC of 4 mg/liter, after which it fell below 90%. The CFR was 6.60%. For the resistance suppression target, the CFR was 5.83%.

At a dose of 4 g a day (Fig. 3A), azithromycin achieved an $EC_{80}$ in 100% of patients up to an MIC of 8 mg/liter. The CFR jumped to 59.71% for optimal microbial kill. However, at that dose the CFR for exposures associated with resistance suppression increased marginally to only 6.60%. Similarly, Fig. 3B shows that with a 6-g dose the $EC_{80}$ target attainment was identical to the 4-g dose up to the MIC of 8 mg/liter; the CFR only increased marginally to 60.22%. Similarly, the CFR for resistance suppression was 6.60%, identical to the 4-g dose. Thus, 6 g offers no major gain compared to 4 g per day.

Figure 4 shows the performance of very high azithromycin doses of 8 g (16 times of standard dose) and 10 g (20 times of standard dose). In Fig. 4A and B, both doses achieved 100% $EC_{80}$ target attainment until an MIC of 16 mg/liter and then fell below 90% above that. This means that even at these very high doses, isolates with MICs of 32 mg/liter are unlikely to be effectively killed and are therefore resistant. For the 8-g dose, the CFR for $EC_{80}$ was 86.80%, whereas that for resistance suppression was 53.67%. The 10-g dose, with CRFs of 86.90 and 59.90%, respectively, did not much improve on that result.

**FIG 3** Target attainment for doses of 4 and 6 g per day. Target attainment rates, and the MICs above which target attainment was 0%, were similar between doses of 4 g a day (A) and 6 g a day (B).

**FIG 4** Target attainment probability for 8 to 10 g. (A) In the case of 8 g per day, target attainment rate fell to 0% at the MIC of 32 mg/liter. (B) The dose of 10 g a day did not improve on 8 g a day.
DISCUSSION

Similar to TB, doses of drugs used to treat pulmonary MAC were chosen out of necessity, which even though in use for decades, it is now clear that many of the original dose choices were suboptimal: PK/PD studies and Monte Carlo simulations have identified larger doses which are now being evaluated in clinical trials (7, 10–12, 29–32). Currently, azithromycin is administered at doses of 500 to 600 mg in combination therapy, based on very limited clinical data, and in the treatment of cavitary MAC pneumonia in the largest multicenter study had a microbial response rate of only 4% (1, 6). Our main finding is that the standard dose is unlikely to achieve optimal microbial kill possible; in fact, it achieved concentrations associated with optimal killing in 0% of patients. Even when the AUC0–24/MIC exposure associated with the minimum amount of microbial kill was used as a target, the standard dose still performed poorly. This poor microbial kill could explain the high failure rates with the current treatment regimens when culture conversion is used to monitor outcome. Unfortunately, even with the high azithromycin doses we tested, a reasonable proportion of patients achieve exposures associated with optimal kill only at doses of 8 g a day. At that dose, an AUC0–24/MIC associated with optimal kill is achieved in 87% of patients, and that associated with resistance suppression is achieved in 54% of patients. However, there is a caveat to our analyses. Our analysis is based on microbial response. Clinical response differs from microbial kill, although it is dependent on it. It has been speculated that azithromycin could also achieve clinical response in patients with bacterial pneumonias via immunomodulation (33, 34). The extent of such immunomodulation, as well as its possible role in pulmonary MAC disease, remains unexplored. On the other hand, it could be that in combination therapy with ethambutol, synergy is encountered, and both microbial kill and resistance suppression could be achieved at low doses. That, however, could just be hopeful thinking, given the poor culture success rates of that regimen at standard doses in the clinic.

Azithromycin therapy is generally safe, and high doses are generally well tolerated. Doses of up to 2 g, as a single dose, are already in clinical use and are highly tolerated. Moreover, high weekly intermittent doses have been used in AIDS patients for many months to years as prophylaxis with good tolerability. Furthermore, in one study, Luke et al. administered intravenous doses of up to 4 g of azithromycin to healthy volunteers, and the maximum tolerated dose limits were not reached (35). Thus, high doses can be administered. Nevertheless, we are not aware of doses higher than 4 g being administered or of doses of 4 g being administered daily. Although reports of azithromycin overdose are thankfully rare, based on a PubMed search, in one case an infant inadvertently received 10 mg of azithromycin/kg and developed life-threatening bradyarrhythmias (36). Thus, caution is needed, and the safety of higher doses would have to be established in the clinic before we can recommend their use. This may also be a point of departure to search for replacements of the macrolides to find pharmacophores that may be more effective against pulmonary MAC disease at more realistic doses. This suggests that there is a need to replace macrolides with drugs that may be better tolerated by patients.

Our second new finding involves the azithromycin susceptibility breakpoint. In general in the treatment of pulmonary MAC, in vitro susceptibility tests have played a limited role in clinical decision-making. A major question is the susceptibility breakpoint, which ideally should separate patients who will respond and those who will not. Correlation between in vitro susceptibility testing for macrolides in MAC and clinical response has been demonstrated in a controlled trial (8). The test is actually performed using clarithromycin susceptibility as the surrogate for azithromycin. For the azithromycin susceptibility itself, resistance is currently defined as an MIC of >256 mg/liter in Middlebrook 7H9 broth (37). Here, we show that even at doses of 10 g a day (if it were possible for patients to tolerate them), there will be no effective microbial kill at MICs above 32 mg/liter. With the standard dose of 500 mg, even if the standard of desirable microbial kill was lowered, and we wanted to achieve any microbial kill whatsoever, no matter how small, and used an EC20 target AUC0–24/MIC of 1.29 mg/liter, the standard dose would still fail to show any microbial killing at an MIC of 4 mg/liter or above. Indeed, a worthwhile exercise is simply to calculate the median and range AUC/MIC achieved by, say, the standard dose, as shown in Fig. 5. At MICs above 4 mg/liter, the AUC falls below the MIC (which is not good), and by 128 mg/liter, one tube dilution below the current standard the AUC/MIC is vanishingly small even if corrected for intracellular penetration ratio. Indeed, the azithromycin susceptibility breakpoints for other respiratory pathogens are also low, in the range of 0.5 mg/liter, which makes more sense given concentrations that can actually be achieved intracellularly in the lung (38). It would be surprising, and inconsistent, if MAC, a pathogen that macrolides have a harder time killing compared to standard community acquired pneumonia related pathogens, had a higher susceptibility breakpoint than these pathogens that azithromycin easily kills (39, 40).

Finally, our findings have implications on the design of regimens in which an agent can suppress resistance to self and not depend on another. It has become very clear in the study of other pulmonary mycobacterial diseases, especially tuberculosis and pulmonary Mycobacterium abscessus infections, that subtherapeu-
tic concentrations drive resistance emergence (23, 41–45). Thus, it is important to design optimal doses upfront that would prevent the emergence of resistance to the drug by itself. This principle has been lacking in the design of new agents in the field of MAC treatment. Indeed, it should be a principal aim when designing new antimicrobial agents that have the potential to treat pulmonary MAC.

Our study has several limitations. First, the azithromycin pharmacokinetics may not be linear, especially at high doses that have not undergone systematic pharmacokinetic analyses. However, at least for doses up to 4 g, the pharmacokinetics are known to be linear (35). In the case of a nonlinear increase in concentration say due to a saturation process, doses higher than 4 g would achieve higher CFR than we calculated. Second, as discussed earlier, azithromycin may have immunomodulatory effects, which we failed to account for. Third, azithromycin pharmacokinetic parameter estimates could be affected by the pulmonary MAC itself, as well as the ethnicity of patients. We used estimates from a large pharmacokinetic study from Japan by Muto et al. involving patients with mubandate bacterial pneumonias (18). However, as part of our second step validation, we compared AUC_{0-7} identified in 240 patients from Denver, CO, treated with 500 mg for pulmonary MAC, which was 1.68 ± 0.95 mg-h/liter, to that in our 10,000 patient simulation, which was 1.51 ± 0.09 mg-h/liter (28). Thus, our assumptions were correct, and the results are generalizable. In addition, the microbial response rates of 0 to 6.6% in the simulations with standard dose were close to the 4% identified in a multicenter study. Thus, our dose and susceptibility breakpoint results likely are clinically relevant.

ACKNOWLEDGMENT

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

REFERENCES


Deshpande et al.


