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Pharmacokinetic and Pharmacodynamic Issues in the Treatment of Mycobacterial Infections

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Abstract The therapy of mycobacterial infections is challenging for a number of reasons. Because mycobacteria are not susceptible to many classes of antibacterial agents, treatment typically requires the use of antimicrobial drugs that are not commonly used and may have small therapeutic windows. For many species, procedures for drug susceptibility testing and optimal treatment regimens have yet to be defined. Finally, because mycobacteria are generally slow to succumb to antimicrobial agents, therapy must be given with multiple drugs for prolonged periods of time, making it necessary to monitor for drug toxicity, drug interactions, and patient nonadherence. Better understanding of the pharmacokinetics and pharmacodynamics of antimycobacterial agents should improve the therapy of mycobacterial infections. Using current treatment strategies for tuberculosis and *Mycobacterium avium* complex infections as examples, this review highlights basic pharmacokinetic and pharmacodynamic principles and the rationale for combination chemotherapy that should also be applicable to other mycobacterial infections.

Introduction

The genus *Mycobacterium* consists of slow-growing, obligate aerobic bacilli with a unique lipid-rich cell wall composition that allows them to take up basic dyes and resist decolorization with acid-alcohol (i.e., to be "acid-fast"). While *Mycobacterium tuberculosis* and *Mycobacterium leprae* are virulent and obligate pathogens, most mycobacteria are denizens of soil and water that are only opportunistic pathogens. Members of the *Mycobacterium avium* complex (MAC), for example, are unlikely pathogens for normal individuals but may cause disease in immunocompromised patients, in persons with abnormal lung anatomy or physiology, or in children.

The therapy of mycobacterial infections is challenging for a number of reasons. Mycobacteria are not susceptible to many classes of antibacterial agents. As a result, mycobacterial infections often require treatment with drugs that are not commonly used for infections with other bacteria and often have small therapeutic windows. In addition, individual species have unique patterns of antimicrobial susceptibility, which necessitates specialized treatment regimens even among the mycobacterial genus. For many species, the optimal treatment regimens have yet to be defined. Finally, because mycobacteria are generally slow to succumb to antimicrobial agents, therapy must be given with multiple drugs for prolonged periods of time, making it necessary to monitor for drug toxicity, drug interactions, and patient nonadherence.

This review is intended to serve as an introduction to the chemotherapy of infections caused by mycobacteria. The specific therapeutic approaches to infections with each of the more clinically relevant mycobacterial species are presented elsewhere in this special section. This overview seeks to use knowledge gained from the treatment of infections caused by *Mycobacterium tuberculosis* and MAC to highlight basic principles of therapy that should be applicable in a more general fashion to many of the mycobacterioses.

Chemotherapy of Tuberculosis

Brief History of the Chemotherapy of Tuberculosis

In 1943, the discovery of streptomycin (SM) and its activity against *Mycobacterium tuberculosis* by Albert Schatz unlocked the door to the antibiotic treatment of tuberculosis (TB) [1]. While monotherapy with SM was able to cure otherwise lethal forms of acute paucibacillary TB such as meningitis and miliary disease, it was soon evident that monotherapy resulted in the selection of drug-

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resistant mutants and treatment failure among patients with multibacillary forms such as cavitary pulmonary TB. In the following years, the discovery of new compounds with antituberculous activity, namely para-aminosalicylic acid (PAS) and isoniazid (INH), ushered in the era of combination therapy. Therapy with SM, PAS, and INH prevented the selection of SM-resistant mutants and resulted in the cure of patients with 18 months of treatment. For more than 20 years, it was the standard treatment for TB.

Beginning in the 1970s, combination therapy with SM, INH, and PAS was progressively replaced by combinations that included INH, rifampin (RIF), pyrazinamide (PZA), and ethambutol (EMB). A remarkable number of well-controlled randomized clinical trials established the efficacy of "short-course" treatment regimens utilizing these agents [2]. These short-course regimens are able to cure multibacillary forms of TB after as little as 6 months of administration and have become the standard of care throughout the world. Still, a number of basic requirements must be met for treatment to be successful: (i) antibiotics must be given in combination to prevent the selection of resistant mutants; (ii) antibiotics must be given for a long period of time, at least 6 months, to prevent relapses after treatment is stopped; and (iii) clinician and patient compliance must be monitored to ensure proper administration and intake of antibiotics. These requirements are often difficult to meet, especially in developing countries and in the context of HIV epidemics. Inadequate therapy leads to poor clinical responses, continued disease transmission, and the emergence and expansion of drug resistance [3, 4]. There is therefore a need for new antituberculous drugs to make treatment easier to implement and to effectively treat drug-resistant disease.

Principles of Chemotherapy for Tuberculosis

First- and Second-Line Drugs for Chemotherapy of Tuberculosis

Numerous antibiotics with antituberculous activity are available, including natural products such as the aminoglycosides, the congeners (SM, kanamycin, amikacin [AMK], viomycin, capreomycin), and cycloserine; synthetic compounds such as the nicotinamide analogs (INH, PZA, and ethionamide) PAS, thiacetazone and EMB; and finally, semisynthetic compounds derived from natural substances such as the rifamycins (RIF, rifabutin [RBT] and rifapentine [RPT]). These agents have long been classified as "first-line" or "second-line" drugs on the basis of their antituberculous activity and toxicity. Because of their potent antituberculous activity and limited toxicity, the drugs INH, RIF, PZA, EMB, and SM are considered first-line agents, while drugs with lesser activity and/or greater toxicity are considered second-line agents and are used primarily in the treatment of patients harboring bacilli resistant to the first-line drugs.

Rationale for Combination Therapy

Combination therapy with at least two drugs active against the infecting organism is necessary to prevent the selection of drug-resistant mutants during therapy, particularly in forms of TB in which the size of the bacillary population is quite large (e.g., cavitary pulmonary TB, in which up to 10^8 organisms may be found) [5]. Infecting tubercle bacilli develop spontaneous chromosomal mutations conferring resistance to single drugs at a predictable frequency ranging from 1 in 10^6, for INH, to 1 in 10^8, for RIF [6]. Thus, if the size of the bacillary population is large enough to harbor drug-resistant mutants, single-drug therapy will eventually lead to selective amplification of the resistant subpopulation. So long as the mechanisms of resistance are independent for drugs given in combination, however, the likelihood of spontaneous resistance to two or more drugs occurring is the product of the probabilities for resistance to each individual agent (e.g., 1 in 10^{14} for INH +RIF). As expected, drugs with increasingly potent bactericidal activity are more effective at reducing the size of the bacillary population and preventing the emergence of resistance to companion drugs. In this regard, INH, RIF, and SM are most effective, EMB is intermediate, and PZA is least effective among the first-line drugs [7].

Duration of Therapy

Antimicrobial drugs must also be given for a long duration of time, at least 6 months, to effectively cure TB. Prolonged therapy is necessary despite relief of symptoms and sputum culture conversion to completely eradicate a small subpopulation of persistent bacilli that have reduced metabolic activity and greater tolerance to the action of antimicrobial drugs. With therapy of inadequate duration, viable "persisters" may cause relapse of clinical disease months or years after apparent cure. For patients with cavitary pulmonary lesions and/or positive sputum cultures despite 2 months of combination therapy, eradication of persisters is even more difficult, and some experts recommend extending the duration of therapy to 9 months.

Two Phases of Therapy

Observations on the activity of individual drugs and combinations in the laboratory and in clinical trials led Canetti [8] to propose the two-phase concept of antituberculous therapy, in which the course of therapy is divided into an initial bactericidal, or "intensive", phase, and a subsequent sterilizing, or "continuation", phase. The vast majority of infecting bacilli are killed during the bactericidal phase (thus reducing clinical symptoms, the risk of transmission, and the emergence of resistance), while the few remaining persisters are eradicated during the sterilizing phase (thus reducing the risk of relapse). Of

great interest is the fact that some drugs have exceptional activity in one phase but not the other [9, 10]. For example, studies of early bactericidal activity of available antituberculous drugs in newly diagnosed patients show INH to have the most potent bactericidal activity [11]. At the same time, INH appears to have relatively poor sterilizing activity. On the other hand, PZA is one of the most effective sterilizing agents despite having minimal early bactericidal activity [10].

The "Special Populations" Hypothesis

On the basis of these and other observations, it has been hypothesized that the growth rate of tubercle bacilli within the infected host varies according to the type of lesion and that the growth characteristics in each lesion result in differing susceptibility to specific antituberculous agents [9, 12]. In a patient with cavitary pulmonary TB, the vast majority of bacilli (i.e., 10^7 or 10^8 organisms) are extracellular and actively multiplying in the liquefied caseous material covering the cavity wall [13]. Under these conditions, the bacilli are readily killed by bactericidal agents (e.g., INH and, to a lesser extent, SM). Bacilli inhabiting solid caseous material, however, are considered semidormant and undergo only intermittent bursts of metabolic activity. These organisms are killed preferentially by RIF, a drug that inhibits transcription of mRNA [14]. Lastly, a small population of organisms is believed to be semidormant within acidic environs, whether intracellular or within areas of active inflammation and recent necrosis. These organisms are particularly susceptible to PZA, a drug that appears to accumulate in tubercle bacilli and exert its activity only under acidic conditions [15, 16]. The latter two subpopulations of bacilli probably number less than 10^5 organisms at the onset of therapy but are believed to be the source of the persisters that require prolonged therapy for eradication.

Potential for Intermittent Administration of Drugs

Despite the relatively short half-life of most antituberculous drugs, combination chemotherapy can be efficacious when given as infrequently as twice a week during the continuation phase of therapy. The so-called "Denver regimen" is popular for directly observed therapy in the USA. It employs daily therapy with INH, RIF, PZA, and EMB for 2 weeks, followed by the same four drugs at higher doses twice weekly for 6 weeks, then INH and RIF twice weekly for the remaining 4 months [17, 18]. Intermittent therapy is possible because of the slow multiplication time of *Mycobacterium tuberculosis* and the prolonged inhibitory action of some drugs that persists even after serum levels fall below the MIC. Intermittent regimens are most successful when preceded by a highly bactericidal intensive-phase regimen that effectively eradicates the rapidly multiplying bacillary population, reducing the risk of treatment failure and selection of drug-resistant mutants. This point is well illustrated by the recent experience with once-weekly continuation-phase regimens using the long-acting rifamycin RPT with high-dose INH. Patients receiving this regimen had a greater risk of treatment failure or relapse when their sputum cultures were still positive at the end of the initial 2-month intensive phase, and relapse with rifamycin-resistant TB has been problematic [19, 20].

Limitations of Current Antituberculous Chemotherapy

When administered appropriately, combination antituberculous therapy can be highly effective anywhere in the world. Regimens employing first-line agents are orally bioavailable, relatively cheap (i.e., $10–$20 through the Global Drug Facility) [21], and generally well tolerated. Cure rates exceeding 85% are possible [22]. However, the discussion in the preceding section serves to highlight several important limitations of current antituberculous therapy. The regimens are lengthy and complex, inviting nonadherence, drug interactions, and drug toxicity. Consequently, to be effective, treatment programs require substantial supervision to monitor adherence and tolerability. Such supervision is often difficult in the developing world [22].

New regimens for TB that could be administered for a shorter duration of therapy or more intermittently (i.e., once weekly or even less frequently) without sacrificing efficacy would reduce the burden of supervising drug administration and make treatment more widely available. Unfortunately, it is difficult to see how existing first-line agents could be used more effectively in this regard, and there are no new agents in the later stages of the drug development pipeline. On a positive note, however, newer fluoroquinolones (i.e., moxifloxacin [MXF] and gatifloxacin) that are currently considered second-line agents offer some promise. MXF has demonstrated potent bactericidal activity and sterilizing activity in vitro [23, 24] and in mouse models [23, 25, 26], suggesting it may be of benefit in shortening the duration of therapy. In addition, MXF's serum half-life of 9–12 hours in humans [27, 28, 29, 30] may make it a better companion drug than INH for once-weekly regimens with RPT [19, 20, 31, 32].

Complex drug-drug interactions, particularly between the rifamycins and antiretroviral agents (e.g., protease inhibitors and non-nucleoside reverse transcriptase inhibitors), may also complicate TB therapy. These interactions are increasingly manageable on the basis of accumulating pharmacokinetic data but will continue to be problematic. In some cases, the use of alternative agents (e.g., RBT) will reduce the impact of these interactions.

For the most part, TB chemotherapy is well tolerated. However, the potential for drug toxicity has recently been highlighted by several reports on the rates of adverse reactions during routine curative treatment [33, 34] and by the unanticipated hepatotoxicity of the 2-month RIF+PZA regimen for latent TB infection in HIV-seronegative patients [35]. This situation is made more difficult by

the poor understanding of the mechanism(s) of action and/or toxicity of PZA. Better understanding of the mechanisms of action and the pharmacodynamics and pharmacogenomics of antituberculous agents should facilitate efforts to reduce the toxicity of TB therapy.

Pharmacokinetics and Pharmacodynamics of Antituberculous Agents

Basic Pharmacodynamic Correlates of Bactericidal Activity

Marked differences in the time course of antimicrobial activity have been demonstrated among the various classes of antibiotics in nonmycobacterial infections. On the basis of experiments conducted in vitro and in animal models, bactericidal activity has been classified as either concentration dependent or time dependent [36]. For drugs with concentration-dependent killing, such as aminoglycosides, rifamycins, and fluoroquinolones, the rate of bacterial killing increases as the concentration of antibiotic increases over a wide range of concentrations. For these antibiotics, the C_{max}/MIC and AUC/MIC ratios, where C_{max} is the maximal serum concentration and AUC is the area under the serum concentration-time curve, correlate best with the rate of killing. On the other hand, for drugs with time-dependent killing, such as beta-lactam agents, the concentration must exceed the MIC of the organism for killing to occur, but the rate of killing does not increase substantially once the concentration increases beyond four to five times the MIC. For these antibiotics, it is rather the time above MIC (T_{MIC}), expressed as the proportion of the dosing interval for which the concentration exceeds the MIC, that correlates best with the rate of killing. Dosing strategies differ for concentration-dependent versus concentration-independent drugs. For the former, the highest possible dosage that does not cause toxicity is favored in order to maximize the drug concentrations (and therefore the rate of killing) at the site of infection. For the latter drugs, however, dosing strategies should maximize the time that drug concentrations exceed the MIC of the organism(s) at the site of the infection.

For antimicrobial drugs exhibiting concentration-dependent activity, such as aminoglycosides and fluoroquinolones, the maximum rate of killing against gram-negative bacilli such as *Pseudomonas aeruginosa* is obtained at C_{max}/MIC ratios of >12, effective bactericidal activity at ratios >8–10, and poor activity at ratios <4 [37]. When utilizing the parameter AUC/MIC for fluoroquinolones, a ratio of ≥100–125 is a reliable predictor of bactericidal activity against the same organisms [38], although maximal bactericidal activity may not be achieved until this ratio exceeds 250 [39]. The magnitude of this parameter does vary for some organisms, however. For example, the magnitude of the AUC/MIC required for bactericidal activity against *Streptococcus pneumoniae* is lower (AUC/MIC ratio, 25–30) than that needed for similar activity against gram-negative bacilli [37, 38].

Since pharmacodynamic parameters can correct for differences in pharmacokinetics between animal species and for differences in antimicrobial susceptibility, the magnitudes of these parameters that are necessary for efficacy against a given pathogen are likely to be similar between different host species and between susceptible and resistant organisms. Therefore, results from animal studies using a suitable model are directly applicable to human infections [36].

The Postantibiotic Effect

Besides describing the relationship between drug concentrations and the rate of bacterial killing, pharmacodynamics also describes the persistence of antimicrobial effects after the drug has been removed [40]. Considered broadly, the latter effect is designated as the postantibiotic effect. It has been used to determine the optimum interval between dosing and is the basis of intermittent twice- or thrice-weekly therapy for TB [41, 42, 43]. INH, RIF, SM, and EMB have each demonstrated postantibiotic effects against *Mycobacterium tuberculosis*. Since concentration-dependent antibiotics tend to have more significant postantibiotic effects [36], infrequent administration of large doses is possible because the prolonged postantibiotic effects protect against bacterial regrowth when serum concentrations fall below the MIC. For this reason, and because drugs that may be given once daily or even less frequently are desirable (and perhaps required) for modern TB chemotherapy, concentration-dependent antibiotics may be most desirable for further development as new antituberculous agents.

Pharmacodynamic Parameters of First-Line Antituberculous Antibiotics in Humans

Due in large part to the wide acceptance of current short-course regimens and the limited number of new drug candidates developed in the last two decades, the pharmacodynamic principles just described for other bacterial infections have not been rigorously studied in regards to the chemotherapy of TB. As a result, it is uncertain whether the same principles described above for fast-growing bacteria apply when treating infections caused by an organism such as *Mycobacterium tuberculosis*, which has a prolonged doubling time, the potential for intracellular replication, and the capacity for dormancy (whether termed latency in the face of host immunity or persistence in the face of antibiotic therapy). Each of these characteristics could significantly alter the relationship between the time course of drug exposure and antimicrobial activity. For example, it is already evident that administration of drugs as infrequently as twice weekly can be successful after as little as 2 weeks of daily intensive therapy. This would not be predicted from the relatively short half-lives of the first-line agents themselves. Secondly, more work is needed to determine if the

Table 1 Pharmacokinetics and pharmacodynamics of INH, RIF and PZA in humans (adapted from Kim et al. [48], Kenny and Strates [47], and Lacroix et al. [49])

Drug	Pharmacokinetic parameters			Pharmacodynamic parameters		
	Dose (mg/kg)	Cmax (μg/ml)	AUC_{24} (mg-h/l)	MIC90 (μg/ml)	Cmax/MIC	AUC/MIC
Isoniazid						
Rapid metabolizers	5	5.4±2.0	19.9±6.1	0.05	108[a]	398[b]
Slow metabolizers	5	7.1±1.9	48.2±1.5	0.05	142[a]	964[b]
Rifampin	10	14.91	117.93	0.25	58.44[a]	471[b]
Pyrazinamide	25	38.7± 5.9	520±101	10	3.8±0.6[c]	52±10[c]

[a]Over 10

[b]Over 125

[c]Below the recommended values

degree to which drugs concentrate intracellularly and kill intracellular bacilli in vitro correlates with bactericidal or sterilizing activity in animal models or clinical studies [44]. Moreover, TB treatment requires combination therapy, and the application of pharmacodynamic principles to drug combinations requires further examination. For example, a recent study suggests that combination therapy prolongs the postantibiotic effect against *Mycobacterium tuberculosis* [45]. Finally, there are no convincing in vitro models to predict the sterilizing activity of antituberculous drugs [44]. Murine models are able to predict sterilizing activity [46], but the necessary experiments are lengthy, labor intensive, and expensive.

Table 1 summarizes the main pharmacokinetic and pharmacodynamic parameters of INH, RIF, and PZA with standard human dosing [47, 48, 49]. The parameters of INH are given separately for patients who are rapid or slow acetylators of the drug. The C_{max} is lower, the half-life shorter, and the AUC smaller in rapid acetylators compared to slow acetylators [50].

With the exception of RIF, it remains to be demonstrated whether each of the major first-line antituberculous drugs kills *Mycobacterium tuberculosis* by concentration-dependent or time-dependent mechanisms. For INH, a single clinical trial using divided dosing suggests that killing may be concentration dependent [51]. In that study, a single daily dose of 400 mg was more effective than 200 mg given twice daily, despite the fact that the former regimen provided INH concentrations above the MIC for only 50–75% of the dosing interval in rapid acetylators, while the latter regimen provided concentrations above the MIC for the entire dosing interval [52]. A plateau in the efficacy of INH has been demonstrated by clinical trials in Madras [51, 53]. No significant differences in outcome were found between slow and rapid acetylators or between patients receiving 400 or 700 mg doses of INH. Such a plateau is similarly suggested by results from a dose-ranging study in which 300 mg and 600 mg doses had similar early bactericidal activity [54]. When considered in the context of achievable C_{max} values for the 300 mg dose of INH (i.e., 3–7 μg/ml) [48, 55] and the MIC90 of INH (0.05 μg/ml), a C_{max}/MIC ratio of roughly 15 would appear to be the threshold for maximal efficacy, similar to target values of this parameter for other concentration-dependent antibiotics against other bacteria. This is further supported by a study in the experimental mouse model in which a daily dose of 25 mg/kg (expected C_{max} of 25–30 μg/ml) had similar activity whether the MIC for the infecting organism was 0.015 μg/ml or 2 μg/ml [56], a finding also suggesting little improvement in bactericidal activity beyond a C_{max}/MIC ratio of 15.

Recent work in the mouse model has shown RIF to have concentration-dependent activity that correlates best with the AUC/MIC ratio [57]. On the basis of an RIF MIC of 1 μg/ml for *Mycobacterium tuberculosis* in serum (which controls for protein binding in vivo), dose fractionation studies in mice revealed that doses expected to achieve an AUC/MIC ratio of 271 in the mouse were associated with a 1 $\log_{10}$ reduction in lung colony-forming unit (cfu) counts after six daily doses. Interestingly, despite their activity, these doses were on the low end of the dose-response curve. Moreover, the AUC/MIC for RIF in humans is expected to be approximately 120 after a 600 mg oral dose [47]. While this value meets the target values for AUC/MIC associated with efficacy for other concentration-dependent antibiotics against gram-negative bacilli, it is clear that higher doses of RIF or more potent rifamycins might exert substantially greater activity. On the other hand, lower doses of RIF appear to be less effective in clinical studies. One randomized clinical trial demonstrated a decline in the activity of RIF with a dose reduction from 600 to 450 mg daily [58]. Increasing the dose to 750 mg did not significantly increase the activity in the same study, although the number of subjects in each group was small. Others have shown that early bactericidal activity is also reduced by reducing the dose from 600 to 300 mg [59]. These findings, together with evidence from the mouse model, suggest that AUC/MIC ratios ≥500 (i.e., AUC_{free}/MIC ratios ≥75–100) are surprisingly close to the threshold for efficacy and suggest that efficacy could be improved if the RIF dosage in humans could be increased without sacrificing safety or tolerability [60].

At first glance, the newly approved rifamycin, RPT, compares favorably with RIF on the basis of its potent in vitro activity against *Mycobacterium tuberculosis* (i.e., MIC in broth = 0.125 μg/ml vs. 0.25 μg/ml for RIF) [61],

Table 2 Pharmacokinetics and pharmacodynamics of rifampin and rifapentine (adapted from Lounis et al. [61])

Drug	$T_{1/2}$ (h)	MIC90 (μg/ml)	C_{max}/MIC	AUC/MIC	C_{max}/MIC[a]	AUC/MIC[a]
Rifampin	2–5	0.25	58	471	8.7	70.8
Rifapentine	14–18	0.125	94	2552	2.8	76.6

[a]Denotes pharmacodynamic parameters calculated using free (unbound) drug concentrations

its prolonged serum half-life in humans (14–18 h vs. 2–5 h for RIF) [62], and its superior pharmacodynamic profile in humans at the approved 600 mg dose (i.e., C_{max}/MIC90 = 94 vs. 60 for RIF, and AUC/MIC90 = 2,552 vs. 472 for RIF) [61]. However, RPT has had somewhat disappointing activity when compared to RIF in three clinical trials of intermittent drug therapy during the continuation phase of TB chemotherapy [19, 20, 32]. A closer look reveals that the exceptional protein binding of RPT (97% vs. 80–85% for RIF) results in markedly reduced free (or active) drug concentrations [62]. Adjustment of the above pharmacodynamic parameters for free drug concentrations reveals a C_{max}/MIC90 of only 2.8 and an AUC/MIC90 of 76.6 for RPT (Table 2), both below the values expected to predict optimal efficacy and below the values for RIF (8.7 and 70.8, respectively). Careful interpretation of these pharmacokinetic/pharmacodynamic data in advance of these trials may have led to more aggressive dosing of RPT and improved efficacy. This claim is supported by the fact that dose increases in the mouse model improved the sterilizing activity of intermittent RPT administration [63]; moreover, larger doses of RPT (e.g., 900–1,200 mg) appear to be well tolerated in humans [64].

There is little known of the pharmacodynamics of PZA in TB. The mechanism of action remains poorly understood and allows no predictions as to whether killing is concentration dependent or time dependent. PZA has no in vitro activity against *Mycobacterium tuberculosis* unless the pH of the medium is reduced to 5.5 or less, a condition in which active *Mycobacterium tuberculosis* replication ceases [65, 66]. It also has poor early bactericidal activity [11] and offers little protection against the selection of resistance to companion drugs in clinical use. The MIC90 is 10 μg/ml in acidified broth. In humans, the C_{max} is 40 μg/ml and the AUC is 520 μg×h/ml [49], giving C_{max}/MIC and AUC/MIC ratios of 4 and 52, respectively. These values are substantiated by the relatively poor bactericidal activity of this dose of PZA in vivo [10].

Pharmacokinetic/Pharmacodynamic Parameters of Fluoroquinolones for *Mycobacterium tuberculosis*

As narrow-spectrum antimicrobial agents whose position was well established before the study of pharmacodynamics became accepted, the drugs INH, RIF, and PZA have not previously been subjected to rigorous pharmacodynamic evaluation. The fluoroquinolones, on the other hand, are among the most studied classes of antimicrobial agents from a pharmacodynamic standpoint. The wealth of research information pertains to the treatment of infections caused by fast-growing organisms, however, and not to chemotherapy of TB. Although first-generation fluoroquinolones have demonstrable activity in human TB [67, 68], the role of this class—in particular in newer, more potent members—has yet to be defined. An in-depth study of fluoroquinolone pharmacodynamics in the experimental chemotherapy of TB would be quite interesting for two reasons. First, the role of the fluoroquinolones in the treatment of human TB may be better defined through better understanding the activity of these drugs against TB in the mouse. Second, their broad spectrum of activity allows more general comparisons to be made between *Mycobacterium tuberculosis* and other bacterial species in terms of the pharmacodynamic parameters and the magnitudes of such parameters that are predictive of activity.

Fluoroquinolones differ from each other in their activity against *Mycobacterium tuberculosis* and their pharmacokinetics in humans (Table 3). The newest molecules, MXF and GAT, have the lowest MIC90 values (0.5 μg/ml) and longest serum half-lives (up to 9–12 h for MXF). The C_{max} values obtained in humans at clinically tolerated doses are typically ≤6 μg/ml, and the C_{max}/MIC90 ratio does not exceed 10 for any of the fluoroquinolones. If this ratio is predictive of bactericidal activity against *Mycobacterium tuberculosis*, as has been shown for the fluoroquinolones against fast-growing bacteria, then the activity of the available fluoroquinolones should be limited, with the

Table 3 Comparative pharmacokinetics and pharmacodynamics of fluoroquinolones after a single oral dose in humans (adapted from Lubasch et al. [30])

Drug	Pharmacokinetics			Pharmacodynamics		
	Dose (mg)	C_{max} (μg/ml)	AUC (μg∗h/ml)	MIC90 (μg/ml)	C_{max}/MIC90	AUC/MIC90
Ciprofloxacin	500 (8.3)	2.4	11.6	1.0	2.4	11.6
Ofloxacin	400 (6.6)	3	24	2.0	1.5	12
Levofloxacin	500 (8.3)	6.2	45	1.0	6.2	45
Sparfloxacin	200 (3.3)	1.1	18.8	0.5	2.2	37.6
Gatifloxacin	400 (6.6)	3.4	30	0.5	6.8	60
Moxifloxacin	400 (6.6)	4.3	39	0.5	8.6	78

exception of MXF and GAT, for which the ratios are in the 7–10 range, which is suitable for effective (though not maximal) bactericidal activity. Considering the ratio AUC_{24}/MIC90, again none of the fluoroquinolones reaches the ideal value of >100–125, desirable for activity against gram-negative bacilli. Only MXF and, to a lesser degree, GAT have values close to 100, suggesting again that these two agents would have stronger anti-TB activity than all other fluoroquinolones at clinically tolerable doses.

Because no comparative studies have been performed in humans, we must turn to the experimental mouse model to ascertain the predictive value of these pharmacokinetic and pharmacodynamic parameters against *Mycobacterium tuberculosis*. Provided that the drug dosages used in the mouse are equipotent to those used in humans (i.e., give similar AUC values), the bactericidal activities of the various fluoroquinolones in the experimental mouse model of TB are generally in agreement with what is predicted from the pharmacokinetic and pharmacodynamic parameters (Fig. 1). MXF, which produces the greatest AUC/MIC in standard human doses, is also the most potent fluoroquinolone against *Mycobacterium tuberculosis* in the murine model [23, 69]. However, the size of the AUC/MIC value achievable in humans, at 70–90, remains below the optimal value of 100–125 demonstrated for efficacy against gram-negative bacilli. It is possible that the pharmacodynamics of the fluoroquinolones are both drug- and pathogen-specific and that, as in *Streptococcus pneumoniae* infections, a minimal AUC/MIC ratio of 30–40 is sufficient to achieve clinical and microbiological success in TB infections [37]. Further work in mouse models, aimed at more precisely simulating the pharmacokinetics of the fluoroquinolones in humans, should help to answer these important questions.

Pharmacodynamics of Antituberculous Drugs, and Prevention of Selection of Drug-Resistant Mutants

Drug resistance in *Mycobacterium tuberculosis* arises through the selective amplification of mutants with spontaneously occurring chromosomal mutations that confer resistance to one or more drugs in use. Selective amplification occurs when the concentration of drug at the site of infection is below the inhibitory concentration for the mutant population and above the inhibitory concentration for the susceptible majority population. Drug concentrations in this "mutant selection window" [70] favor the growth of the mutant population over that of the susceptible population, whereas concentrations above or below the window provide no selective advantage. For example, Dong et al. [70] have recently demonstrated that a static fluoroquinolone concentration above the MIC for organisms with any first-step mutation in the target enzyme DNA gyrase (a.k.a. the "mutant prevention concentration") will prevent the selection of resistant mutants despite prolonged antibiotic exposure. Unfortunately, it is not possible to achieve sustained fluoroquinolone concentrations above the mutant prevention concentration in humans. In fact, only a few of the available fluoroquinolones (e.g., MXF and GAT) are even able to achieve a C_{max} above the mutant prevention concentration. It would, therefore, be more clinically relevant to determine target values for pharmacodynamic parameters (perhaps even novel pharmacodynamic parameters incorporating the mutant prevention concentration or time within the mutant selection window) that correlate with the prevention of mutant selection.

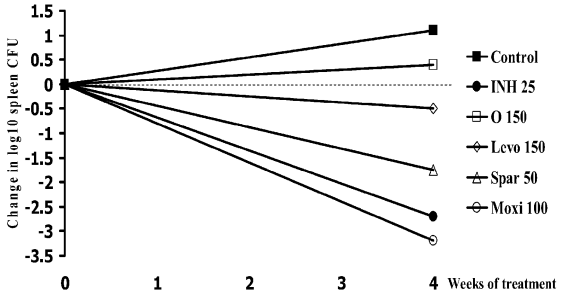

Fig. 1 Bactericidal activity of fluoroquinolones against *Mycobacterium tuberculosis* in the mouse model of TB. Isoniazid (INH) was used as a positive control. The fluoroquinolones are ofloxacin (O), levofloxacin (Levo), sparfloxacin (Spar), and moxifloxacin (Moxi). Drugs were given once daily by gavage 5 days/week. The dosages are given in mg/kg body weight. The figure is adapted from Ji et al. [23, 69]

Because clinically significant resistance is believed to occur most often through stepwise mutations, the fluoroquinolones are the most appropriate class for the study of mutant prevention parameters with single-drug therapy. In contrast, single-point mutations confer levels of resistance to INH, RIF, PZA, and SM that cannot be overcome by achievable drug concentrations, meaning that monotherapy will reliably select resistant organisms, provided the initial bacterial population is large enough to allow for such a mutant to arise spontaneously.

Combinations of drugs used for intermittent therapy can also be studied for their ability to prevent the selection of resistant mutants. As in the example of once-weekly INH/RPT regimens, the use of drug combinations in which individual drugs do not have the same half-life will produce periods of functional monotherapy that may result in the selection of resistant mutants [20, 31]. In vitro pharmacodynamic systems in which the exposure of *Mycobacterium tuberculosis* cultures to drug combinations whose concentration-time profiles mimic those in humans may provide a means for gauging the likelihood of the selection of resistant mutants before preclinical and clinical testing.

Role of Therapeutic Drug Monitoring

In general, the current short-course regimens for TB are highly effective and well tolerated. Under circumstances in which the risk of treatment failure is judged to be higher than normal, however, therapeutic drug monitoring (TDM) to ensure appropriate serum drug concentrations may

assist clinical decision-making [71]. In a recent example, low serum concentrations of INH were associated with treatment failure, relapse, and/or selection of rifamycin resistance among patients treated with a once-weekly regimen of INH/RPT in the continuation phase [31]. Although the benefit of prospective TDM was not assessed in this study, one might speculate that the identification of low INH concentrations in patients at increased risk of treatment failure or in those slow to respond to therapy could have led to more aggressive dosing of INH or a change to a regimen with more frequent drug administration.

As for general recommendations, patients with persistent symptoms (fever, weight loss, night sweats, cough) and sputum smear positivity after the first 1–2 months of therapy may benefit from TDM. Similarly, given the reliance of antituberculous drugs on renal (EMB, SM, cycloserine) or hepatic (INH, RIF, PZA, ethionamide, PAS) clearance, there should be a low threshold to perform TDM for patients with renal or hepatic failure who are experiencing a poor clinical response or signs of toxicity. TDM may be particularly useful for patients with HIV infection who are treated concomitantly with antiretroviral drugs that inhibit or induce hepatic microsomal enzymes (e.g., protease inhibitors and non-nucleoside reverse transcriptase inhibitors); in such patients, TDM can verify adequate drug delivery for the antituberculous as well as the antiretroviral agents. Lastly, TDM should be used in patients under treatment for multidrug-resistant TB because many second-line drugs have narrow therapeutic windows; thus, TDM may help prevent further selection of resistant organisms.

Chemotherapy of Infections with *Mycobacterium avium* Complex

Species belonging to *Mycobacterium avium* complex (MAC) may cause either chronic pulmonary disease in patients without well-defined immunologic disorders or disseminated infection in immunocompromised patients, predominantly in the HIV-seropositive population. Since many of the general principles for treatment are similar, the forms of disease will be considered together.

Principles of Chemotherapy for Infections with *Mycobacterium avium* Complex

Establishment of a Definitive Diagnosis

In contrast to *Mycobacterium tuberculosis*, MAC is a ubiquitous environmental inhabitant that has the potential to contaminate clinical specimens and colonize nonsterile anatomical sites as well as cause invasive disease. Because therapy of MAC infection is lengthy and involves multiple medications with substantial toxicity and complex drug interactions, it is important that treatment be limited to those patients demonstrated to have invasive disease. Cultures from blood or other sterile sites (e.g., lymph node, bone marrow) will generally suffice for the definitive diagnosis of disseminated MAC infection. However, because MAC may colonize the airways without causing disease, criteria have been established to determine the clinical significance of MAC isolated from respiratory specimens [72]. Bacteriologic diagnosis should be made on the basis of at least three sputum specimens demonstrating three positive cultures with negative smears or three positive cultures with at least one positive smear. If sputum is unobtainable, a single positive culture from a bronchial wash specimen is sufficient, provided there is 2+ or greater growth or a positive smear. Clinical and radiographic criteria must also be satisfied for a definitive diagnosis of pulmonary MAC infection [72].

Macrolides as the Cornerstone of Therapy

In contrast to the therapy of TB, which is nearly uniformly effective when administered correctly, the therapy of MAC infections has historically been less efficacious. This owes to the fact that MAC has greater intrinsic antibiotic resistance and a predilection for patients with pre-existing lung disease and/or profound immunosuppression. Before the introduction of clarithromycin and azithromycin for the treatment of MAC infection, the outcome of therapy was often unsuccessful. For pulmonary MAC infection, rates of sustained clinical response were below 50%, despite the use of up to six drugs, and relapse after discontinuation of therapy was all too common [73, 74, 75]. Treatment durations of 1 year or more were routinely required to obtain sputum culture conversion, leading to problems with toxicity and nonadherence to therapy.

The situation with disseminated MAC infection was similarly difficult. During the first decade of the HIV epidemic, patients routinely died before clinical or microbiological efficacy could be demonstrated. As the overall medical treatment of patients with AIDS improved, three- and four-drug regimens that included EMB, a rifamycin (RIF or RBT), clofazimine, INH, or ciprofloxacin were shown to clear bacteremia and lead to symptomatic improvement in some patients [76, 77], but adherence and toxicity remained problematic.

The second-generation macrolides, clarithromycin (CLA) and azithromycin (AZI), have revolutionized the treatment of MAC disease and now represent the cornerstone of MAC chemotherapy. Both drugs have in vitro activity against MAC (CLA MICs are generally 4 μg/ml or less), are highly concentrated intracellularly, and have exceptional tissue penetration. Macrolide-containing regimens achieve more rapid sputum and blood culture conversion and are associated with lower relapse rates [78, 79, 80, 81]. Moreover, they are administered orally and are generally well tolerated. Failure to respond to a macrolide-containing regimen is usually a consequence of macrolide resistance, nonadherence to therapy, or drug intolerance. Uncontrolled data suggest AZI may be modestly less

effective than CLA in the treatment of pulmonary MAC infection, but both macrolides are clearly efficacious [82].

Rationale for Combination Therapy

As in the treatment of TB, combination therapy is intended to capitalize on the additive and/or synergistic effects of antimycobacterial agents and prevent the selection of drug-resistant mutants. Additive and/or synergistic effects are demonstrable with the addition of EMB to the macrolides in vitro [83]. The rifamycins also have additive and/or synergistic activity when added to EMB [84]. The benefit of combination therapy has been shown in the mouse model of disseminated MAC infection and in human trials.

Data from Mouse Experiments

To date, the beige mouse is the most widely used animal model for experiments of MAC infection and treatment. It is more susceptible to MAC infection than is the immunocompetent mouse [46], presumably due to a deficiency of natural killer cells [85]. Using such a model, the anti-MAC activities of monotherapy with CLA, AZI, RIF, RBT, AMK), EMB, sparfloxacin, and clofazimine were compared [86, 87]. CLA and, to a lesser degree, AZI had dose-dependent bactericidal activity. The activity of CLA, for example, increased over a range from 50 to 100 to 200 mg/kg, equipotent to 500, 1,000, and 2,000 mg per day in humans, respectively. In addition, CLA in the beige mouse model demonstrated similar activity against different strains of MAC, suggesting natural susceptibility of MAC to CLA [88]. Both RIF and RBT were inactive. AMK and EMB displayed modest bactericidal activity similar to that of CLA at 100 mg/kg. Sparfloxacin and clofazimine had bacteriostatic effects [87].

In the beige mouse model, the prevalence of CLA-resistant mutants is approximately 1 per 10^8 organisms prior to treatment. CLA monotherapy resulted in the progressive selection of CLA-resistant mutants on the condition that the treatment began when the population of organisms in the spleens was greater than 10^7 [86, 89]. As in the treatment of TB, combination therapy, preferably with at least two bactericidal agents, is necessary to prevent the selection of drug resistance. Two-drug combinations of CLA with minocycline, EMB, or RBT did not prevent the selection of CLA-resistant mutants (Fig. 2). Even the three-drug combination of CLA, EMB, and RBT was unsuccessful. The proportion of CLA-resistant mutants isolated did not differ significantly from that observed in mice treated with CLA alone. The prevention of CLA resistance was obtained only with the combination of CLA and AMK, when AMK was given for at least 4 weeks [89].

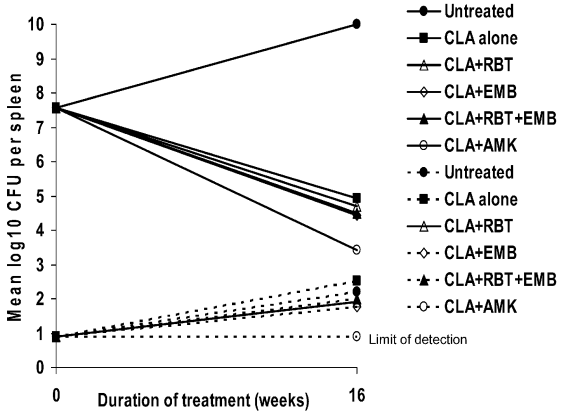

Fig. 2 Number of total colony-forming units (cfu) and clarithromycin-resistant mutants in spleens of untreated control and treated mice. Beige mice were inoculated intravenously with $10^{6.7}$ cfu of a *Mycobacterium avium* complex strain 101. Treatment began after 4 weeks and was given for a total of 16 weeks. All drugs were given by gavage 6 times weekly at the following dosages (in mg/kg): CLA 200, RBT 10, EMB 125, AMK 100. Solid lines represent total cfu counts. Dotted lines represent cfu counts for clarithromycin-resistant mutants. The figure is adapted from Lounis et al. [89]

Data from Clinical Studies

Data from clinical studies confirm both the efficacy of macrolide monotherapy in pulmonary and disseminated MAC infection and the potential for selection of macrolide-resistant mutants [90] with resultant clinical failure [91]. When added to a macrolide, the combination of EMB plus RBT appears to provide better protection than EMB alone against the development of resistance in patients with disseminated MAC infection [92].

Initial Susceptibility Testing of Mycobacterium avium Complex: Helpful Only for the Macrolides

Susceptibility testing of MAC has yet to be standardized. While in vitro susceptibility testing for CLA and AZI correlates with the clinical response to therapy [93, 94], the results for other drugs are less than reliable. For example, EMB resistance is a natural feature of *Mycobacterium avium* species, and susceptibility testing with EMB is not helpful for guiding the therapy of macrolide-naïve patients [95]. For this reason, susceptibility testing is not recommended for guiding the choice of initial therapy [72]. Susceptibility to macrolides should be assessed when patients fail to improve within 6 months or have recrudescent symptoms with positive cultures after consecutive negative cultures. It should also be assessed when patients develop disseminated infection while taking a prophylactic macrolide regimen. The benefit of retaining a macrolide in a treatment regimen despite in vitro resistance is unclear, but macrolide resistance has been associated with clinical failure when macrolides are the only active agent in the regimen [91]. For patients who have received previous treatment, are failing therapy, or cannot tolerate the first-line agents, in vitro susceptibility testing for drugs other

than the macrolides, when performed in an experienced laboratory, may be an important element of management [83].

Adverse Effects and Drug Interactions

CLA commonly causes nausea and abdominal discomfort, while AZI may be better tolerated. EMB is usually well tolerated but may cause retrobulbar neuritis. Because the macrolides and EMB are renally cleared, both may carry a greater risk of toxicity in the elderly (including auditory dysfunction from macrolides). RBT holds an advantage over RIF in terms of anti-MAC activity but is associated with an increased incidence of adverse effects, namely uveitis, polymyalgia, and leukopenia. Theoretically, the use of intermittent treatment regimens could reduce the frequency of drug intolerance, but this has not been the experience thus far [96].

Drug interactions can be particularly complex in the HIV-infected patient on concomitant antiretroviral therapy. Two-way interactions between the protease inhibitors and non-nucleoside reverse transcriptase inhibitors and the rifamycins and between CLA or fluconazole and RBT can make management particularly complicated. In general, RBT significantly induces the metabolism of nelfinavir, indinavir, saquinavir, and delavirdine. RBT metabolism is, in turn, inhibited by amprenavir, nelfinavir, indinavir, ritonavir, delavirdine, CLA, ciprofloxacin, and the azole antifungal agents. On the other hand, RBT metabolism is induced by nevirapine and efavirenz. Expert recommendations should be followed when RBT is used together with any of the drugs listed above [97]. The potential clinical impact of the induction of CLA metabolism by RBT on the activity of CLA remains to be elucidated, although the three-drug regimen of CLA, EMB, and RBT is clearly efficacious. No dosage adjustment for CLA is currently recommended.

Prophylaxis Against Disseminated *Mycobacterium avium* Complex Disease

Because their unique susceptibility increases dramatically when their CD4+ lymphocyte counts are below 50/mm^3, patients with AIDS should be offered prophylactic therapy to protect against disseminated MAC infection [97]. There is new significance to this recommendation, since macrolide prophylaxis is associated with survival benefit and may allow time for immune reconstitution with highly active antiretroviral therapy [98]. Once CD4+ cell counts are stable above 100/mm^3 for ≥3 months on highly active antiretroviral therapy, primary MAC prophylaxis may be discontinued [97].

Effective regimens include the following: once-weekly AZI (1,200 mg), twice-daily CLA (500 mg), daily RBT (300 mg), or once-weekly AZI plus daily RBT [94, 98, 99, 100]. The macrolides are favored because of their greater efficacy [94, 100, 101] and more limited drug interactions and because of the theoretical risk of promoting rifamycin monoresistance in patients with active TB who receive MAC prophylaxis with RBT [97]. Macrolides also confer additional protection against respiratory bacterial infections as well as *Pneumocystis* pneumonia [102]. While there has been no head-to-head comparison between the macrolides, AZI is generally favored because it may be given once weekly, is better tolerated, and has fewer drug interactions. Among patients who developed MAC bacteremia while receiving prophylaxis with macrolides, macrolide-resistant isolates were isolated from 16% of those treated with AZI and from 29–58% of those treated with CLA [94, 98, 101]. Interestingly, rifamycin resistance occurs less commonly after failure of prophylaxis with RBT, which suggests poor adherence to therapy, poor absorption, or poor activity of RBT as the reason for failure [100].

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