



Review

Prospects for Development of New Antimycobacterial Drugs, with Special Reference to a New Benzoxazinorifamycin, KRM-1648

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Abstract. In this article, I have thoroughly reviewed the status of development of new antimycobacterial drugs, in particular, rifamycin derivatives (rifabutin, rifapentine, and a new benzoxazinorifamycin, KRM-1648), fluoroquinolones (ciprofloxacin, ofloxacin, sparfloxacin, levofloxacin, gatifloxacin, sitafloxacin, moxifloxacin, and others), new macrolides (clarithromycin, azithromycin, roxithromycin), and others. In this review, I have mainly described the *in vitro* and *in vivo* activities of these drugs against *Mycobacterium tuberculosis* and atypical mycobacteria, especially *Mycobacterium avium* complex. In addition, therapeutic efficacy of these drugs in cases of clinical treatment of mycobacterial infections have also been briefly mentioned.

Key words: mycobacterial infection; *Mycobacterium tuberculosis*; *Mycobacterium avium* complex; rifamycin; fluoroquinolones; new macrolides.

Introduction

The resurgence of tuberculosis (TB) in industrialized countries and the worldwide increase in the prevalence of atypical mycobacterial infections in immunocompromised hosts have prompted the quest for new antimycobacterial drugs. The appearance of multidrug-resistant (MDR) strains of *Mycobacterium tuberculosis*, which exhibit *in vitro* resistance to at least two

major antituberculous drugs (usually isoniazid (INH) and rifampin (RMP)) and cause intractable TB, has greatly contributed to the increased incidence of TB³. For instance, well-documented outbreaks or mini-epidemics of MDR-TB in the U. S. have indicated extremely rapid progression of this disease with an associated high rate of mortality, particularly in HIV-infected patients. At present, the most effective therapy for TB is the use of the most active antituberculous drugs, such

Abbreviations used: TB – tuberculosis, MDR – multidrug-resistant, MAC – *Mycobacterium avium* complex, INH – isoniazid, RMP – rifampin, PZA – pyrazinamide, EB – ethambutol, SM – streptomycin, RBT – rifabutin, RPT – rifapentine, CFZ – clofazimine, AMK – amikacin, RXM – roxithromycin, CAM – clarithromycin, AZM – azithromycin, CPFX – ciprofloxacin, KM – kanamycin, OFLX – ofloxacin, SPFX – sparfloxacin, LVFX – levofloxacin, GPFX – grepafloxacin, PZFX – pazufloxacin, PUFX – prulifloxacin, GFLX – gatifloxacin, STFX – sitafloxacin, MFLX – moxifloxacin, KRM – KRM-1648.

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as INH, RMP, pyrazinamide (PZA), ethambutol (EB), and streptomycin (SM), in combination, and a cure rate approaching nearly 100% can be obtained for drug-sensitive TB, using multidrug regimens such as INH + RMP + SM (or EB) and INH + RMP + PZA + SM (or EB). However, because of the recent emergence of strains of MDR – *M. tuberculosis*, some of which exhibit significant resistance to all known antimycobacterial drugs, the development of potent new antituberculous drugs without cross-resistance with known antimycobacterial agents is urgently needed.

Mycobacterium avium complex (MAC) infections, in particular *M. avium* infections, are frequently encountered among patients with AIDS in the U. S., European countries, and other nations. MAC infections in AIDS patients are disseminated rather than restricted to the lungs, presumably due to serious deterioration of CD4 (helper/inducer) T cell function in AIDS patients, resulting in severe suppression of the antimicrobial functions of macrophages, which are crucial effector cells in the host defense mechanisms against mycobacterial infections. Thus, the most common clinical manifestation in MAC-infected patients with AIDS is mycobacteremia, and in these patients pulmonary MAC disease is rare. Clinical management of MAC infections, especially those in AIDS patients, is very difficult, since many drugs are largely ineffective for such infections, for the following reasons⁴. First, MAC infections are frequently encountered in immunocompromised hosts including HIV patients. Second, MAC has intrinsic resistance to the majority of common antituberculous drugs due to its impermeability to these agents. Moreover, the range of susceptibilities of MAC isolates to most antimicrobials, but not to macrolides, is extremely broad. Third, polyclonal MAC infections in AIDS patients may contribute significantly to the lack of success in treating MAC infections. The development of new antimicrobials with potent anti-MAC activity is thus urgently desired. In this review, I will mainly describe the *in vitro* and *in vivo* antimycobacterial activities of the principal classes of new antimycobacterial drugs, including some rifamycin derivatives, fluoroquinolones, and new macrolides.

Rifamycin Derivatives

Rifamycins are among the most important anti-*M. tuberculosis* agents. A number of rifamycin derivatives^{5, 8, 10, 12}, including rifabutin (RBT; ansamycin, LM-427), rifapentine (RPT; DL-437, MDL-437), SPAS-565

(CGP40/469A), R-76-1, FCE-22250, FCE-22807, CGP-7040, CGP-27557, and CGP-29861, have been synthesized. RBT is now included in drug regimens for the treatment of TB and other mycobacterial infections in AIDS patients. As determined by MIC, RBT is about 4 to 6 times more active than RMP against *M. tuberculosis*, although partial cross-resistance exists between RBT and RMP. Moreover, RBT possesses favorable pharmacokinetic features such as a long half-life and good tissue penetration. RPT is also much more active than RMP *in vitro*, and has pharmacokinetic properties better than those of RMP, such as long half-life. This drug is in phase II trials as a new component of multidrug regimens for the treatment of TB. As described below, a new benzoxazinorifamycin, KRM-1648, is about 100 times more active than RMP against *M. tuberculosis* and exhibits markedly potentiated therapeutic efficacy against *M. tuberculosis* and MAC infections induced in mice. This drug is in phase II stage of development.

In vitro studies of RBT and RPT have revealed the following. First, *in vitro* activity against *M. tuberculosis* and MAC is in the order RBT > RPT > RMP. There is cross-resistance for *M. tuberculosis* and MAC among these drugs, although it is not complete. These drugs are inactive against *M. fortuitum*, *M. abscessus*, and *M. chelonae*. Second, potentiation of *in vitro* activity has been observed with combinations of RBT with either EB, clofazimine (CFZ), amikacin (AMK), or roxithromycin (RXM). In this context, the findings of the recent study by YAJKO et al.¹¹ are most interesting. They evaluated the *in vitro* anti-*M. avium* bactericidal activities of a total of 132 two-, three-, and four-drug combinations including RBT (0.5 µg/ml), clarithromycin (CAM) (2.7 µg/ml), azithromycin (AZM) (0.5 µg/ml), ciprofloxacin (CPFX) (2.6 µg/ml), EB (4 µg/ml), CFZ (0.7 µg/ml), and AMK (20 µg/ml) using drug concentrations that are achievable in serum (C_{max}) at routine dosages. When the mean survival of 10 strains of *M. avium* was measured after 7-day incubation in Middlebrook 7H9 medium with test drug combinations, 12 combinations of drugs were found to yield decreases in CFU of ≥ 2.0 log units with more than 90% of test strains (2 three-drug and 10 four-drug combinations), and 9 of these 12 highly active drug combinations involved RBT: RBT + CAM + EB + AMK, RBT + EB + CPFX + AMK, RBT + CAM + AZM + AMK, RBT + CAM + CPFX + AMK, RBT + CAM + CFZ + EB, RBT + CAM + AZM + CPFX, RBT + CAM + EB + CPFX, RBT + CAM + EB, and RBT + EB + AZM + CPFX, in order of magnitude of activity. Similarly, there were a total of 60 combinations yielding decrease

in CFU of ≥ 1.0 with more than 90% of strains (1 two-drug, 17 three-drug, and 42 four-drug combinations), and 34 of these 60 highly or moderately active drug combinations involved RBT. These findings clearly indicate the superior activity of RBT in potentiating the anti-MAC microbicidal activity of multidrug regimens. YAJKO et al.¹¹ also reported that many combinations involving RBT, such as RBT + CAM + CFZ + EB, RBT + CAM + EB + AMK, RBT + CAM + CFZ, RBT + CFZ + EB + AZM, RBT + CAM + CPFX, RBT + CAM + EB, were strongly active in killing intracellular *M. avium* in J774 cells, a mouse macrophage-like cell line.

In vivo studies using mouse experimental infection models have revealed the following. First, RBT displayed appreciable therapeutic efficacy against murine infections due to *M. tuberculosis* and MAC. RBT exhibited potential efficacy in prophylaxis of tuberculosis in mice. The therapeutic efficacies against MAC infection of some rifamycins were determined as to be in the order RBT $\geq$ RPT $>$ RMP, with RBT as effective as CAM. Therapy of MAC-infected mice with RBT (or in some studies with RPT) in combination with either EB, CFZ, EB + CFZ, CAM + EB, AZM, kanamycin (KM), or CFZ + KM was more effective than that with each drug alone.

In clinical studies, RBT were effective against intractable TB and MAC infections (positive response: 8–92%). Efficacy was achieved with certain regimens involving RBT (RPT) in combination with other drugs, including EB, CFZ, CAM, INH, and CPFX. Moreover, RBT is useful for prophylaxis of AIDS-related MAC infections.

Fluoroquinolones

A number of fluoroquinolones^{1, 5, 12}, such as ofloxacin (OFLX), enoxacin (ENX), CPFX, lomefloxacin (LFLX), tosfloxacin (TFLX), fleroxacin (FLRX), sparfloxacin (SPFX), temafloxacin (TMFX), levofloxacin (LVFX), nadifloxacin (NDFX), grepafloxacin (GPFX), balofloxacin (BLFX), pazufloxacin (PZFX), prulifloxacin (PUFX), gatifloxacin (GFLX; AM-1155), sitafloxacin (STFX; DU-6859a), moxifloxacin (MFLX; BAY12-8039), and others have been or are being developed. Among these quinolones, OFLX, CPFX, and LVFX may be promising for the treatment of TB, particularly MDR-TB. The incidence of mycobacterial resistance to fluoroquinolones is relatively low at present, and there are no reports of cross-resistance or antagon-

ism with other classes of antimycobacterial drugs. Fluoroquinolones can be administered orally with good absorption and favorable pharmacokinetics, including extremely efficient penetration into tissues and host macrophages. Moreover, the incidence and severity of adverse effects are generally low for the fluoroquinolones. Thus, fluoroquinolones may be used for long-term therapy of tuberculosis patients, especially those with HIV infection, in combination with other antimycobacterial drugs.

In vitro studies of the antimycobacterial activities of various quinolones have revealed the following. Fluoroquinolones exhibit fairly potent anti-*M. tuberculosis*, anti-*M. kansasii*, and anti-*M. fortuitum* activities, and the *in vitro* anti-*M. tuberculosis* activities of new quinolones, as determined by their MICs, are in the order STFX $\approx$ GFLX $\approx$ MFLX $\approx$ SPFX $>$ LVFX $\approx$ CPFX $>$ OFLX. Newly synthesized quinolones, such as WQ-3034 and HSR-903, had activity against *M. tuberculosis* comparable to that of LVFX, and newly developed quinolones, including GPFX, PUFX, PZFX, and T-3811ME (des-F(6)-quinolone), had anti-*M. tuberculosis* activity comparable to or less than that of OFLX. Notably, PD161148, a new 8-methoxyquinolone, as is MFLX, exhibits potent activity against CPFX-resistant *M. tuberculosis* due to *gyrA* (gyrase) gene mutation. In our recent study, the antimicrobial activities of fluoroquinolones against *M. tuberculosis* residing within human macrophages were in the order SPFX $\approx$ STFX $>$ LVFX $\approx$ WQ-3034 $>$ HSR-903 $>$ CPFX. Fluoroquinolones exhibit combined activity against *M. tuberculosis* in the following combinations: OFLX+ (EB, INH, PZA) and OFLX + RMP + INH. Although fluoroquinolones also exhibit efficacy against MAC in combination with EB, INH, CFZ, AMK, RMP, and RBT, their anti-MAC activities are relatively weak.

Fluoroquinolones exhibited fairly good *in vivo* activity against experimental mouse infections due to *M. tuberculosis*, *M. kansasii*, and *M. fortuitum*. Their therapeutic efficacies against *M. tuberculosis* infection were in the order MFLX $\approx$ SPFX $\approx$ LVFX $>$ OFLX $>$ pefloxacin. In addition, appreciable efficacy of fluoroquinolones, including CPFX, OFLX, and SPFX, was demonstrated in clinical studies of treatment of MDR-TB and disseminated MAC infection in AIDS patients, particularly when they were included in multidrug regimens. The multidrug regimens OFLX + PZA, CPFX + (RMP, PZA), and (OFLX, CPFX) + RMP + INH yielded 50 to 100% positive response in clinical control of intractable tuberculosis. Fluoroquinolones are also efficacious in clinical treatment of *M. fortuitum* infections.

New Macrolides

Recent clinical trials of MAC therapy for AIDS-associated *M. avium* infections have revealed the following. Although some drugs such as new macrolides^{2, 4-6, 12} (especially CAM) and EB have been found to be significantly efficacious in treating MAC patients, other ordinary drugs are either not efficacious or have not yet been clearly established to be efficacious. New macrolides, including CAM, AZM, and roxythromycin (RXM), have relatively strong *in vitro* anti-MAC activity, and are quite efficacious in treating disseminated MAC infections in AIDS patients, causing a marked decline in MAC counts in blood. For instance, CAM produced an approximately 3 log decrease in CFU counts in *M. avium* blood cultures in cases of disseminated *M. avium* disease. Moreover, CAM decreases clinical symptoms of MAC infection such as fever and night sweats. However, since resistance develops in 15 to 46% of patients following CAM monotherapy, combination of CAM with other drugs is recommended. Notably, new macrolides are also highly efficacious in preventing MAC disease in AIDS patients, when used prophylactically. Although AZM has a half-life of 195 h in alveolar macrophages, which is much longer than that of CAM (4 h), it does not exceed the therapeutic efficacy of CAM against MAC infection in humans and experimental animals, in terms of decreasing blood CFU count.

In vitro studies of CAM, AZM, and RXM have indicated the following. These new macrolides have fairly potent anti-MAC and anti-*M. kansasii* activities, while they are inefficacious against *M. tuberculosis* and *M. abscessus*. Their *in vitro* antimycobacterial activities, as determined by MICs for MAC, are in the order CAM > RXM > AZM > erythromycin (EM). CAM has MICs for MAC isolates comparable to those of SPFX and RMP. New macrolides exhibit activity in combination against MAC in multidrug regimens including (CAM, RXM) + (RMP, INH, EB, PZA), (CAM, RXM) + EB + (RMP, RBT), CAM + INH + RMP + EB, CAM + RBT + EB + AMK, and others. Notably, the *in vitro* activity of new macrolides tends to decrease in acidic pH.

New macrolides exhibit fairly good *in vivo* activity against experimental MAC infection of mice, while they are inefficacious against *M. tuberculosis* infection. Their therapeutic efficacies against MAC infection are in the order CAM ≥ RXM > AZM. CAM is more efficacious against MAC infection than are EB, SM, INH, and OFLX. New macrolides exhibit effects against MAC infection in combinations with other drugs in-

cluding CAM + (RMP, RBT, CFZ), AZM + (RBT, RPT), CAM + (RBT, CFZ, KM) + EB, CAM + INH + AMK, CAM + RMP + EB + CFZ, and others.

Excellent therapeutic efficacy of CAM and AZM has been reported in cases of clinical treatment of disseminated MAC infection in AIDS patients and those with intractable pulmonary MAC infections. In cases of disseminated MAC infection, these macrolides effectively eliminated the organisms from blood of patients with relatively low incidence of adverse effects, even when patients were given monotherapy with these macrolides. However, it should be emphasized that monotherapy with macrolides frequently resulted in the emergence of macrolide resistance. Multidrug regimens containing CAM, such as CAM + EB, CAM + CFZ, and CAM + EB + (INH, CFZ, RBT), are fairly efficacious in treating AIDS-associated disseminated MAC infections (positive response: 54–69%). According to the guidelines of the American Thoracic Society (ATS), multidrug regimens containing CAM (or AZM), such as CAM (AZM) + RMP (RBT) + EB + (SM) and CAM (AZM) + EB + (RBT), are recommended for the treatment of HIV-negative pulmonary MAC infection and AIDS-associated disseminated MAC infection, respectively. Moreover, CAM and AZM are also efficacious in preventing disseminated MAC infections in AIDS patients. In the ATS guidelines, regimens of CAM or AZM alone or RBT + AZM are recommended for prophylaxis of disseminated MAC infection, in addition to RBT alone.

KRM-1648^{7, 9, 10}

A new benzoxazinorifamycin, KRM-1648 (KRM) with the unique chemical structure 3'-hydroxy-5'-(4-alkyl-1-piperazinyl) benzoxazinorifamycins (alkyl residue: isobutyl) has been synthesized in Japan. This drug has much more potent antimycobacterial activity than other rifamycin derivatives, including RMP, RBT, and RPT. Phase II clinical studies of KRM are underway in the U. S.

In vitro activity

KRM exhibits excellent *in vitro* activity against slowly growing mycobacteria including *M. tuberculosis* and MAC. On the basis of MIC, KRM is 16 to 512 times more active against these organisms than RMP, and 2 to 8 times more active than RBT. Similar

results were obtained with a radiometric method using the BACTEC 460 TB system. Correlational analysis of MICs for KRM for individual isolates of *M. tuberculosis* and those for RMP and RBT indicated the presence of cross-resistance in most organisms to KRM and RMP and to KRM and RBT. In our recent study of the mechanism of the *in vitro* antimycobacterial activity of KRM, this drug displayed a level of inhibitory activity against RNA polymerase of *M. avium* similar to that of RMP, but exhibited markedly increased permeability into mycobacterial cytoplasm through the cell wall and/or cell membrane.

KRM exhibits strong antimicrobial activity against intramacrophagial *M. tuberculosis* and MAC, causing rapid killing of the organisms when added at the C_{max} . KRM is more efficacious than RMP and RBT in exerting microbicidal activity against *M. tuberculosis* and MAC residing in macrophages, principally because of its extremely low MICs for these organisms. The strong efficacy of KRM is also due in part to the high ratios of intracellular accumulation of KRM, which were 40- to 70-fold higher than that found for RMP. However, MICs of KRM against intramacrophagial *M. tuberculosis* were comparable to its MICs against extracellular *M. tuberculosis*. It thus appears that, after uptake of KRM by macrophages, only a portion of it was delivered to *M. tuberculosis* organisms replicating within macrophages. It should also be noted that the activity of KRM against intramacrophagial *M. tuberculosis* was greatly decreased when RMP-resistant strains were used as target organisms.

KRM has significant bactericidal effects against MAC, and its efficacy against MAC is much greater than that of CAM. We recently found that the bactericidal activity of KRM against intramacrophagial MAC is potentiated by combined use with EB, but only slightly potentiated by CAM. Although KRM effectively killed intramacrophagial MAC, its activity was decreased when MAC organisms resided in Type II alveolar epithelial cells.

In vivo activity

KRM has high tissue levels but low plasma levels in mice after oral administration, as is also the case for RBT, whereas RMP has high levels in both plasma and tissues. AUCs were in the order KRM > RMP > RBT for lungs, KRM > RBT $\approx$ RMP for spleen, RMP > KRM > RBT for liver, RMP > KRM > RBT for kidney, and RMP > KRM $\approx$ RBT for plasma. Moreover, KRM had a longer time of elimination from tissues than did RMP

and RBT, particularly in lungs and spleen, and exhibited non-linear kinetics of elimination. These findings indicate that the tissue distribution properties of KRM are comparable to those of RMP and superior to those of RBT.

KRM has much greater *in vivo* therapeutic efficacy than RMP and RBT against mycobacterial infections induced in animals including mice and rabbits. Although clinical isolates of *M. tuberculosis* exhibited cross-resistance between KRM and RMP or between KRM and RBT, KRM still exhibited significantly stronger *in vivo* activity against moderately RMP-resistant *M. tuberculosis* strains than did RMP. KRM may therefore be useful for clinical treatment of patients with *M. tuberculosis* infection due to strains with moderate levels of RMP resistance. KRM given at long intervals such as once per two weeks exhibited therapeutic efficacy against *M. tuberculosis* infection comparable to those of RMP given at much shorter intervals such as twice weekly. KRM may thus also be useful for treatment of tuberculosis patients who cannot be administered anti-tuberculous drugs at short intervals because of severe adverse drug effects or for other reasons. Moreover, KRM can substitute for RMP in multidrug regimens for tuberculosis treatment, since some studies have demonstrated combined effects of KRM with other agents such as INH and EB in treating *M. tuberculosis* infections in mice.

KRM exhibited stronger *in vivo* therapeutic activity than RMP and RBT against MAC infections in beige and BALB/c mice and rabbits. Although KRM alone did not completely eliminate MAC organisms at sites of infection in mice, some multidrug combinations of KRM with other agents including CAM, CFZ, and SM yielded steady elimination of organisms from the spleens of *M. avium*-infected mice. This suggests that multidrug regimens involving KRM may sometimes be useful for clinical control of MAC infections. It should be emphasized that KRM rapidly caused negative conversion of blood culture of organisms in rabbits with disseminated *M. avium* infection and completely eliminated MAC organisms from sites of infection including the lungs, spleen, liver, and kidneys. This suggests that KRM may be useful in treating the disseminated MAC infections frequently encountered in AIDS patients. Thus, KRM may be added to the list of antimycobacterial drugs that can be recommended for treatment of MAC disease after completing a series of clinical studies. In order to evaluate the usefulness of KRM for clinical control of tuberculosis and MAC infections, a Phase I study was begun in the U. S. in 1995, and a Phase II study is now underway.

Conclusion

In this article, I have thoroughly reviewed the status of development of new antimycobacterial drugs. There are a number of difficulties in drug-design for development of new drug formulations with increased potential for antimycobacterial effects, excellent pharmacokinetics, and tolerability. Also, further efforts are required to improve the properties of the rifamycin derivatives, fluoroquinolones, and new macrolides as antimycobacterial drugs for clinical use. Other types of agents with appreciable levels of antimycobacterial activity have also been reported. In particular, 2-pyridone (ABT-255) is a promising anti-*M. tuberculosis* drug, since its MICs for RMP-resistant isolates of *M. tuberculosis* are very low (0.016 to 0.032 µg/ml) and it exhibits high therapeutic efficacy against experimental mouse infection with RMP-resistant *M. tuberculosis*. Moreover, it has recently been demonstrated that mefloquine, an antimicrobial agent widely used for the prophylaxis of chloroquine-resistant *Plasmodium falciparum* malaria, has MICs of 8 to 16 µg/ml for MAC organisms and is efficacious against MAC infection induced in mice. Since these drugs appear to have promising properties as antimycobacterial drugs, further detailed studies of them should be performed.

It should be emphasized that the most urgent goal of chemotherapy of tuberculosis and MAC infections, especially that associated with HIV infection, is to develop highly active, low-cost drugs which can be used not only in industrialized countries but also in developing countries, since the incidences of AIDS-associated intractable tuberculosis and disseminated MAC infection are now rapidly increasing in the latter.

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