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Activity of Quinolones Against Mycobacteria

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Abstract

The fluoroquinolones have been shown to be active *in vitro* against many mycobacterial species, including most strains of *Mycobacterium tuberculosis* complex and *M. fortuitum*, and some strains of *M. kansasii*, *M. avium-intracellulare* (MAI) complex and *M. leprae*. Ciprofloxacin, ofloxacin and sparfloxacin are the best studied of these agents to date, and are among the most active of this group against *M. tuberculosis* and other mycobacteria. Treatment of patients with multidrug-resistant pulmonary tuberculosis using ofloxacin has resulted in the selection of quinolone-resistant mutants in a few patients. Many strains of MAI, however, are resistant to fluoroquinolones, and structure-activity relationships and DNA gyrase studies have been undertaken to identify the moieties associated with activity and the lack thereof. The genetic and molecular basis of quinolone resistance in mycobacteria has revealed both the recent progress made in these areas and the limitations of the quinolones against this genus. Considerable progress will need to be made in resolving these issues in order for the quinolones to become clinically useful antimycobacterial agents.

During the past decade, the synthesis of new 4-quinolone-3-carboxylates and the evaluation of these agents for antibacterial activity has continued, and the role of various DNA gyrases has been more clearly defined. The most active representatives of these fluoroquinolones include ciprofloxacin, sparfloxacin, moxifloxacin, trovafloxacin and sitafloxacin (DU-6859a).

The fluorinated quinolones show excellent activity *in vitro* against *Mycobacterium tuberculosis*, *M. kansasii*, *M. xenopi*, and *M. fortuitum*. Activity against *M. avium-intracellulare* (MAI) complex is, however, method- and strain-dependent. Approximately 40% of MAI strains are susceptible to quinolones, with ciprofloxacin and sparfloxacin being the most active quinolones currently in clinical use. The development of quinolones with increased activity against MAI is an important goal, towards which progress has been made.

1. Clinical Relevance

No new antituberculosis agents have been developed since the introduction of rifampin, so fluoroquinolones have been investigated for potential efficacy in tuberculosis. *In vitro* studies have shown that fluoroquinolones are active against *M. tuberculosis* at achievable concentrations and treatment studies in mice have demonstrated efficacy. Few clinical studies have been performed in humans, but ciprofloxacin has demonstrated significant early bactericidal activity. Regimens including quinolones for tuberculosis have been shown to be equivalent to standard antituberculosis regimens, and these agents are currently suggested for the management of multidrug-resistant infections, or in patients with adverse reactions to other agents.[1] However, the outcome of regimens including quinolones has been poorer in HIV-seropositive patients. Treatment recommendations for nontuberculous mycobacterial respiratory infections generally do not include quinolones, except for selected infections due to rapid growers.[2] Ciprofloxacin and ofloxacin are the quinolones most often evaluated and recommended in mycobacterial diseases.[3]

2. *In Vitro* Activity

MIC_{90} values of ciprofloxacin against *M. tuberculosis* have been reported to vary from 0.2 to 4 mg/L, while values for sparfloxacin were 0.2 to 0.5 mg/L, levofloxacin 0.25 to 4 mg/L, clinafloxacin 0.25 to 2 mg/L, sitafloxacin 0.2 mg/L, moxifloxacin 0.5 mg/L, and trovafloxacin > 8 mg/L (table I).[4-8]

The activity of quinolones against nontuberculous

Table I. MIC_{90} values (mg/L) of selected quinolones against *Mycobacterium tuberculosis*

Reference no.	Ciprofloxacin	Clinafloxacin	Levofloxacin	Ofloxacin	Sparfloxacin	Trovafloxacin	Sitafloxacin (DU-6859a)	Moxifloxacin
4	0.25-4	0.25-2	0.25-4		0.06-1			
5				0.2	0.2		0.2	
6	4		2		1	>8		
7		1			0.5			0.5

mycobacteria was more variable, with agents such as sitafloxacin having the lowest MIC values against *M. kansasii* and *M. fortuitum*, while the activity of all agents against *M. avium* group and *M. chelonae* was poor (table II).[4,8]

3. Intracellular Activity in Macrophages

The ability to inhibit the *in vitro* growth of mycobacteria within human monocytes was studied with a panel of new quinolones against 2 strains of *M. avium*.[9] 16 compounds with MIC_{90} values ranging from 2 to >32 mg/L were evaluated in a 7-day monocyte assay, using ciprofloxacin as the comparator. The degree of inhibition of intracellular growth correlated with the MIC values. PD 139586, PD 143289, PD 135144, PD 119421 and PD 131575 were the most active agents, with activities superior to those of ciprofloxacin and sparfloxacin. Intracellular activity was shown to correlate well with MIC data.

The intramacrophage activity of ofloxacin and pyrazinamide has also been evaluated. A clinically achievable level of pyrazinamide enhances the antimycobacterial effect of low, non-bactericidal levels of ofloxacin and does not impede the bactericidal effect of a higher, clinically effective level of ofloxacin. Unlike the combination of pyrazinamide and rifampin, these effects are not affected by the sequence of drug administration. These findings support the use of these agents as a preventive combination therapy for individuals exposed to multidrug-resistant *M. tuberculosis*.[10]

4. Structure-Activity Relationships (SAR)

A series of SAR studies has been performed using *M. avium* and other mycobacteria. In a SAR study of N-1-tert-butyl-substituted quinolones, 45 of the quinolones tested were active against the *M. avium-M. intracellulare* complex, with MIC_{50} values <32 mg/L. 24 of these quinolones had activities equivalent to, or greater than, that of ciprofloxacin, and 9 of them had activities equivalent to, or greater than, that of sparfloxacin. The most active compounds were the N-1-tert-butyl-substituted quinolones, PD 161315 and PD 161314, with MIC_{50} values of 0.25 mg/L and MIC_{90} values of 1 mg/L; comparable values for ciprofloxacin were 2 and 4 mg/L, respectively, while for sparfloxacin they were 1 and 2 mg/L, respectively.[11] These values show that the tert-butyl substituent is at least as good as cyclopropyl in rendering high levels of antimycobacterial activity. However, none of the quinolones showed activity against ciprofloxacin-resistant laboratory-derived *M. avium-M. intracellulare* complex strains.

Another study evaluated a series of quinolones with substitutions at the C8 position, to examine the relationship between structural modifications at this position and activity against mycobacteria.[12] The compounds were evaluated for their activities against *M. fortuitum* as a measure of *M. tuberculosis* activity. The results demonstrated that the contribution of the C8 position to antimycobacterial activity was dependent on the substituent at N1 and was in the order: (i) COMe = CBr > CCI > CH ≡ CF= COEt > N > CCF3, when N1 was cyclopropyl; (ii) N ≡ CH > CF > COMe, when N1 was 2,4-difluorophenyl; (iii) N ≥ CH, when N1 was tert-butyl; and (iv) N > CH, when N1 was ethyl. In general, derivatives with piperazine substituents at C7 were slightly less active against mycobacteria than analogues with pyrrolidine substituents, regardless of the pattern of substitution at the C8 position.

Evaluation of a series of N1- and C7-substituted quinolones was performed to examine specific SAR between modifications of the quinolone at these 2 positions and activity against mycobacteria.[13] Compounds were evaluated for activity against *M.*

Table II. MIC_{90} values (mg/L) of selected quinolones against nontuberculosis mycobacteria

Strain	Ciprofloxacin	Clinafloxacin	Levofloxacin	Ofloxacin	Sparfloxacin	Sparfloxacin	Sitafloxacin (DU-6859a)
Reference no.	3	3	3	5	3	5	5
Mycobacterium kansasii	8	>8	4	3	2	3	0.8
M. avium group	>8	>8	>8	50	8	12.5	12.5
M. fortuitum	4	2	4	3.3	2	1.6	0.4
M. chelonae	>8	>8	>8	>100	>8	>100	6.3

fortuitum and *M. smegmatis* as a barometer of *M. tuberculosis* activity. The results of this study demonstrated that (i) the activity against mycobacteria was related more to antibacterial activity than to changes in the lipophilicity of the compounds, (ii) the antimycobacterial activity imparted by the N1 substituent was in the order tert-butyl ≥ cyclopropyl > 2,4-difluorophenyl > ethyl ≡ cyclobutyl > isopropyl, and (iii) substitution with either piperazine or pyrrolidine heterocycles at C7 afforded similar activity against mycobacteria.

5. Genetic Basis of Quinolone Activity in Mycobacteria

DNA gyrase and DNA topoisomerase IV, collectively referred to as the bacterial type II DNA topoisomerases, are the targets of quinolones.[14] DNA gyrase is an A2B2 complex encoded by *gyrA* and *gyrB* genes, while DNA topoisomerase IV is a C2E2 complex encoded by *parC* and *parE* genes. In Gram-negative bacteria, DNA gyrase is the primary and DNA topoisomerase IV is the secondary target of ciprofloxacin, while the inverse is the case for Gram-positive bacteria. Mutations in the quinolone resistance-determining region (QRDR) of the enzyme, the primary target for a quinolone, results in resistance. Characterisation of these enzymes in mycobacteria and in resistant mutants allows correlation of genotype and susceptibility phenotype.

In a study of the *in vitro* activities of 7 quinolones and the sequences of the QRDRs in the A and B subunits of DNA gyrase for 14 mycobacterial species, quinolone activity could be ranked from greatest to least activity as follows: sparfloxacin > levofloxacin > ciprofloxacin > ofloxacin > pefloxacin > flumequine > nalidixic acid.[15] Based on MIC values, the mycobacterial species could be organised into 3 groups: resistant [*M. avium, M. intracellulare, M. marinum, M. chelonae, M. abscessus* (ofloxacin MIC values, ≥8 mg/L)], moderately susceptible [*M. tuberculosis, M. bovis BCG, M. kansasii, M. smegmatis* (ofloxacin MIC values, 0.5 to 1 mg/L)], and susceptible [*M. fortuitum, M. peregrinum* (ofloxacin MIC values, ≤0.25 mg/L)]. Peptide sequences of the GyrB QRDR were identical in all the species, including the amino acids at the 3 positions known to be involved in acquired quinolone resistance, i.e. 426 (Asp), 447 (Arg), and 464 (Asn) [numbering system used for *Escherichia coli*]. The last 2 residues could be involved in the overall low level of mycobacterial susceptibility to quinolones, since they differ from those found in the very susceptible *E. coli* (Lys-447 and Ser-464) but are identical to those found in the less susceptible species, *Staphylococcus aureus* and *Streptococcus pneumoniae*. Peptide sequences of the GyrA QRDR were identical in all the mycobacterial species, except for the amino acid at position 83, which was an alanine in the 2 less susceptible groups and a serine in the most susceptible one, as in *E. coli*. This suggests that this amino acid is also involved in the observed differences of quinolone susceptibility within the *Mycobacterium* genus.

The mechanism of resistance to fluoroquinolones in *M. tuberculosis* was studied by selecting spontaneous fluoroquinolone-resistant mutants from a susceptible strain, H37Rv, and testing the susceptibilities of these mutants and 2 fluoroquinolone-resistant clinical isolates (A-382, A-564) to various fluoroquinolones.[16] The QRDR sequence of *gyrA* was also determined in this study. Spontaneous fluoroquinolone-resistant mutants of H37Rv appeared at frequencies of 2×10^{-6} to 1×10^{-8}. For 3 mutants selected on ciprofloxacin, ofloxacin, and sparfloxacin, respectively, and the 2 clinical isolates, MIC values of ciprofloxacin and ofloxacin were as high as 16 mg/L, and those of sparfloxacin were 4 to 8 mg/L. Cross-resistance was displayed to all fluoroquinolones evaluated. Sparfloxacin and FQ-A (PD 127391-0002) were the most potent fluoroquinolones tested. All of the fluoroquinolone-resistant strains (MIC values, ≥4 mg/L) had mutations in the QRDR, which led to substitution of the Asp residue at position 87 (Asp-87) by Asn or Ala, or the substitution of Ala-83 by Val, in the A subunit of DNA gyrase. Similar mutations have been noted in other bacterial species.

Characterisation of the mechanisms of resistance to fluoroquinolones by *M. tuberculosis* was determined in another study.[17] Mutants of strain H37Ra were selected *in vitro* with ofloxacin and their QRDRs for *gyrA* and *gyrB* were amplified and sequenced to identify mutations in gyrase A or B. Three types of mutants were obtained: (i) a mutant (TKp1) that had no mutations in *gyrA* or *gyrB*; (ii) mutants that had single missense mutations in *gyrA*, and (iii) mutants that had 2 missense mutations, resulting in either 2 altered gyrase A residues or an altered residue in both gyrases A and B. The TKp1 mutant had a 2- to 4-fold increase in the MIC values of ofloxacin, ciprofloxacin, and sparfloxacin. Gyrase mutations caused a much greater increase in the MIC values of fluoroquinolones. For mutants with single *gyrA* mutations, increases in the MICs were 4- to 16-fold, and for mutants with double gyrase mutations, the MICs were increased 32-fold or more. A *gyrA* mutation in TKp1 secondary mutants was associated with a 32- to 128-fold increase in the MIC values of ofloxacin and ciprofloxacin and an 8-fold increase in the MIC of

sparfloxacin. Sparfloxacin was the most active fluoroquinolone tested, and no sparfloxacin-resistant single-step mutants were selected. High-level sparfloxacin resistance (i.e. MIC values ≥5 mg/L) was associated with 2 gyrase mutations. Mutations in *gyrB* and possibly altered levels of intracellular accumulation of drug are 2 additional mechanisms that may be used by *M. tuberculosis* in the development of fluoroquinolone resistance. Because sparfloxacin is more active *in vitro* and selection of resistance appears to be less likely to occur, it may have an important advantage over ofloxacin or ciprofloxacin for the treatment of tuberculosis.

A multidrug efflux pump has also been described in mycobacteria.[18] This mechanism was associated with the presence of the *lfrA* gene, cloned from chromosomal DNA of quinolone-resistant *M. smegmatis* mc2-552. The gene conferred low-level resistance to fluoroquinolones when present on multicopy plasmids. Sequence analysis suggested that *lfrA* encoded a membrane efflux pump of the major facilitator family. This study suggested that the LfrA polypeptide encoded for by the *lfrA* gene catalyses the active efflux of several quinolones.

6. Conclusions

This review of the *in vitro* activity of quinolones, the SAR of quinolones, and the genetic and molecular basis of quinolone resistance in mycobacteria, has revealed both the recent progress made in these areas and the limitations of the quinolones against this genus. Considerable progress will need to be made in resolving these issues for the quinolones to become clinically useful antimycobacterial agents.

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