Analysis of dual active fluoroquinolones in *Streptococcus pneumoniae*

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Sir,

A recent review by Smith et al.1 and associated correspondence2,3 raised important unresolved issues regarding which quinolones exert dual activity through gyrase and topoisomerase IV in *Streptococcus pneumoniae*. Dual activity involves the substantial engagement of both enzyme killing pathways and is of particular interest in potentially limiting the emergence of resistance. Smith et al.3 concur with us2 that genetic studies are very important in identifying dual active drugs and agree that clinafloxacin is one such agent.1 Our original assignment of clinafloxacin as the archetypal dual action quinolone was based on genetic criteria:4 (i) that gyrA or parC resistance mutations each had minimal (~two-fold) effects on clinafloxacin MICs but higher level resistance was seen for gyrAparC mutants, and (ii) that gyrA mutants could be selected with drug but only at the MIC and at low frequency (10−9 to 10−10). These findings are consistent with both gyrase and topoisomerase IV contributing substantially to drug action in vivo. We wish to point out that gemifloxacin, gatifloxacin and moxifloxacin share these same features5,8 and therefore, based on the clinafloxacin paradigm, should also be considered as dual active.

The view of Smith et al.3 that gemifloxacin, gatifloxacin and moxifloxacin are not dual active derives from two arguments involving laboratory strains and clinical isolates, both of which are problematic. In the case of laboratory strains, Smith et al.3 report their unpublished observations that gyrA or parC mutants can be obtained by challenge with each of the quinolones. They comment that such mutants should not be recoverable (for dual action drugs) based on our suggestions ‘that with clinafloxacin, gatifloxacin, gemifloxacin and moxifloxacin, there is no increase in MIC observed with *S. pneumoniae* isolates that have either a parC or gyrA mutation alone’ and that ‘the mutants must have mutations in both gyrA and parC’. We do not make the attributed statements.5 Indeed, as with clinafloxacin, it is well known that either a gyrA or a parC change confers a small (~two-fold) increase in MIC for gemifloxacin, gatifloxacin and moxifloxacin.5,8 Presumably, even when both targets are comparably engaged by a dual active drug, a mutation in one target increases the drug concentration needed to register drug effects through the other target, e.g. by requiring increased cleavable complex levels.

Moreover, as with clinafloxacin,4 it is documented that gemifloxacin, gatifloxacin and moxifloxacin select single-step gyrA mutants displaying an ~two-fold MIC increase.5,8 Recovery of gyrA (rather than parC) mutants may indicate that growth inhibition through gyrase is marginally more favoured for these agents. At all events, the crucial issue for dual activity is that parC and gyrA mutations have similarly small effects on MICs and that mutants are recovered only in a narrow drug concentration range (at or near the MIC), at low frequency, and exhibit minimal resistance. At least from published accounts, these features are met by gemifloxacin, gatifloxacin and moxifloxacin.5,8 The unpublished work of Smith et al.3 selecting single gyrA mutants ‘highly resistant’ to gatifloxacin and moxifloxacin and of parC mutants with gemifloxacin is intriguing but will require evaluation in the context of other studies. Interestingly, further support for dual activity comes from the finding that S79F ParC or S81F GyrA mutations reduce enzymic DNA cleavage induced by clinafloxacin, gemifloxacin, gatifloxacin and moxifloxacin by some 8– to 64-fold in vitro (see Ref. 2, K. A. Gould and L. M. Fisher, unpublished results) but in terms of resistance in vivo, these effects are moderated to ~two-fold by drug action on the other target.

The second argument of Smith et al.3 against dual activity is based on the MICs of resistant *S. pneumoniae* clinical isolates, but is unpersuasive. By directly correlating quinolone MICs with *parC*gyrA status, they conclude that a single *parC* mutation gives a spread of MIC increases to gatifloxacin and moxifloxacin in clinical strains, and moreover, that the presence of a single gyrA mutation results in dramatic MIC increases. Unfortunately, clinical isolates are heterogeneous and may carry multiple resistance mutations that confound the interpretation of MICs. Therefore, large MIC increases observed for clinical strains cannot be confidently attributed wholly to *parC* or *gyrA* alterations and used to argue against dual activity. Though we agree it is very important to understand quinolone resistance arising in the clinical setting,4 there are difficulties in drawing unambiguous conclusions about drug action from such studies. Stepwise-selected laboratory mutants bearing well-characterized resistance mutations will continue to play a key role in guiding the analysis of dual activity and other mechanistic aspects of quinolone action.

References


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Sir,

The continued correspondence with Fisher et al.1,3 regarding our recent review of the dual activity of fluoroquinolones2 highlights controversial and important aspects of this topic. The serious issue of increasing worldwide antibiotic resistance has generated significant interest in dual-active fluoroquinolones. Dual-active fluoroquinolones demonstrate comparable activity in both cellular targets: DNA gyrase and topoisomerase IV.5 As we have previously stated,4 and Fisher et al.1,3 have reiterated, a fluoroquinolone demonstrates dual activity if a single mutation in gyrA or parC has a minimal (two-fold) effect on the MIC, and that it requires mutations in both gyrA and parC to generate substantially increased MICs (≥4-fold). Based on this definition, clinafloxacin can be considered a dual-active fluoroquinolone.6 Conversely, we do not believe that the current data on gatifloxacin, gemifloxacin and moxifloxacin indicate that these agents are dual-active fluoroquinolones. The basis of this belief is results from clinical strains2,5 and our current work-in-progress laboratory strains. Fisher et al.1,3 state that results based on clinical and laboratory strains are problematic, and only well-defined isogenic laboratory strains should be used for dual-activity studies. Ideally, all research would be carried out with well-defined laboratory strains. However, results can only be applied in the context in which they were created and should not be extrapolated beyond those limitations. It is thus essential to evaluate the activity of fluoroquinolones on clinical isolates, as those are the results which are truly indicative of the situation in which the fluoroquinolones are to be employed.

Fisher et al.1,3 indicate that the crucial issue surrounding the dual activity of fluoroquinolones is whether parC or gyrA mutations will have little effect on the MIC, or whether mutants are recovered only at, or near, the MIC at low frequency. Based on these ideas, our laboratory mutants demonstrate that gatifloxacin, gemifloxacin and moxifloxacin are not dual active.9 Significant strain variability is apparent, and has resulted in the intermittent recovery of mutants at significantly increased MICs (8–16-fold).9 These mutants appeared at frequencies of 10−5 and 10−6.9 Sequencing of quinolone-resistance determining regions (QRDRs) of parC and gyrA was carried out subsequently with these mutants. The mutants that were recovered with high frequency at fluoroquinolone concentrations significantly elevated from their initial MIC had only single mutations in parC or gyrA.9 Thus, mutations in both parC and gyrA are not required to develop an elevated MIC.

In response to the second disagreement of Fisher et al.,1,3 as the clinical isolates were collected from the clinical environment in which fluoroquinolones are used, we feel data based on these isolates are highly significant and persuasive. In addition to sequencing the QRDRs of parC and gyrA, the clinical isolates on which we based our conclusions that parC mutations increase the MICs of gatifloxacin and moxifloxacin and that single gyrA mutations dramatically increase the MIC were also serotyped, genotyped and evaluated for efflux mechanisms.9 These are well-characterized clinical isolates. It is imperative that dual-active fluoroquinolones be evaluated with clinical isolates in order minimize treatment failures. If a single parC mutation had little effect on the MIC of dual-active fluoroquinolones then these agents could be used to treat infecting organisms with a single mutation. This is not the case, and no fluoroquinolone should be used for an S. pneumoniae infection with a known parC mutation.10 It is vital that prescribing physicians are not misled into believing that supposed dual-active fluoroquinolones can be used to treat intermediate- or low-level resistant organisms containing a single mutation.10 Davidson et al.11 recently published a report on fluoroquinolone treatment failure that resulted from the administration of levofloxacin for the treatment of an organism with a parC mutation. There are no data to suggest that gatifloxacin or moxifloxacin would have resulted in bacteriological cures in this case. To infer that they would be bacteriologically effective is misleading, and microbiologically and clinically unproven.

We agree that well-defined laboratory strains are essential in the evaluation of quinolone action and activity. However, it is essential that dual activity be studied in the clinical setting in order to find out how the fluoroquinolone will function in a true treatment situation.

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