

Usual and unusual antibacterial effects of quinolones

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Recently documented antibacterial effects of quinolones are reviewed. DNA gyrase is most likely to be the primary target site for these agents. Quinolones rapidly kill susceptible bacteria; the mechanisms of the bactericidal activity, still poorly understood, probably involve new protein synthesis. Quinolones alter membrane integrity before cell death, leading to leakage of cytoplasmic constituents. In Gram-negative bacteria, quinolones act as chelating agents for outer membrane divalent cations, disorganizing the bacterial lipopolysaccharide layer and facilitating the further entry of quinolone molecules in a 'self-promoted' pathway. Quinolones inhibit plasmid replication and reduce the efficacy of plasmid conjugation. Subinhibitory concentrations of quinolones can interfere with bacterial virulence factors, such as bacterial adherence to the host cell, phagocytosis and production of enzymes implicated in virulence. Recent studies also indicate synergism of quinolones with oxacillin against methicillin-resistant staphylococci and describe improved activity of newer compounds against Gram-positive pathogens.

Introduction

Development of quinolone agents has been impressive during recent years, accounting for their wide use in clinical practice. These drugs have shown a remarkable potency against usual pathogens, as well as against bacteria that are less common and difficult to treat, including multiply resistant nosocomial bacteria and various intracellular pathogens. How quinolones act against these numerous organisms, however, is not completely understood. This review will focus on some recently discovered antibacterial effects of quinolones.

Quinolones inhibit DNA gyrase functions

DNA gyrase is an enzyme capable of introducing negative supercoils into bacterial DNA, and responsible for the accommodation of the long circular chromosome within the limited space provided by the cell envelope. The principal prerequisite for the various and complex activities of the enzyme is its ability to cleave and rejoin double-stranded DNA.

Most of the multiple biochemical functions of DNA gyrase on cell physiology are affected as a result of the quinolone activity, including the inhibition of DNA replication, post-replicative repair (Priel, 1984), recombination of DNA and separation of the two circular daughter chromosomes in the terminal phase of replication (Smith, 1986; Sutcliffe, Gootz & Barrett, 1989). Yet exactly how quinolones interfere with DNA gyrase function remains unclear or controversial. Do quinolones exert their effect by binding to DNA gyrase? Such a hypothesis can be supported by several observations: inhibition of the enzymatic activity of purified DNA gyrase by quinolones

(Barrett, Sutcliffe & Gootz, 1990), bacterial resistance associated with DNA gyrase mutations (Gellert *et al.*, 1977; Sato *et al.*, 1986) and ^{19}F nuclear resonance experiments showing the affinity of pefloxacin for DNA gyrase (Le Goffic, 1985). However, if quinolones bind directly to DNA gyrase, the exact binding site remains to be determined. Other reports have suggested that quinolones bind to DNA, and not directly to DNA gyrase (Shen & Pernet, 1985; Tornatelli & Pedrini, 1988) or to the tertiary DNA gyrase-DNA complex (Shen *et al.*, 1989). As a final remark, a computer program, matching experimental data pertaining to DNA gyrase inhibition with quinolone structures predicted with acceptable accuracy the consequences of structural variations for MICs (Klopman *et al.*, 1987): DNA gyrase is indeed a primary target for the quinolones.

Killing of bacteria by quinolones: a puzzling question

Since DNA gyrase inhibition blocks bacterial replication, the expected effect of quinolones would be bacteriostatic. In fact, exposure of a susceptible bacterial population to a quinolone results generally in rapid cell death, followed by a plateau at a viable cell count about 10^{-4} -fold below the initial inoculum (Wolfson, Hooper & Swartz, 1989). Bactericidal activity can even be shown during the stationary phase of growth (Zeiler, 1985).

The mechanisms of these lethal events remain unclear. Killing is reduced, paradoxically, by increasing the quinolone concentration over a certain level and is also markedly inhibited by adding rifampicin or chloramphenicol (Crumplin, Kenwright & Hirst, 1984). Rifampicin, chloramphenicol and high quinolone concentrations have in common the capacity to inhibit protein synthesis suggesting that the killing activity by quinolones is associated with new protein synthesis.

Quinolones and SOS response

One possible explanation for the bactericidal activity is that quinolones cause DNA gyrase to damage DNA (Smith, 1984), inducing the SOS DNA repair system. Cell-damaging environmental conditions trigger the synthesis of specific cascades of proteins, whose physiological role is to repair the affected cellular system, and protect it from further damage. Among other phenomena, this SOS response enhances DNA repair capacity and mutagenesis and blocks cell division (producing filamentation) and cell respiration (Little & Mount, 1982). The quinolones were found to trigger the SOS response, with a maximum intensity at the quinolone concentration that produced the greatest bactericidal effect (Phillips *et al.*, 1987). In SOS mutants however, quinolone killing did not correlate necessarily with the SOS phenotype; furthermore induction of the SOS response was protective to fleroxacin and enoxacin treated bacteria, but not to ciprofloxacin treated bacteria (Walters, Piddock & Wise, 1989). Taken in aggregate, these experiments dealing with the SOS response do not close the discussions about the killing, but suggest that the mechanisms of action of different quinolones are not uniform in their precise detail, as was originally thought.

Loss of membrane integrity

Electron microscope studies showed that quinolone treated *Escherichia coli* cells displayed areas of reduced cytoplasmic contrast at the time when cell death occurred

(Dougherty & Saukkonen, 1985). At the same time, the cells became more susceptible to detergent, and exhibited increased membrane permeability. These quinolone-induced changes were attributed to the loss of membrane integrity, leading to leakage of cytoplasmic constituents, and, ultimately, cell death (Dougherty & Saukkonen, 1985). Again in *Esch. coli*, quinolones induced lipopolysaccharide release and increased cell surface hydrophobicity (Chapman & Georgopapadaku, 1988). The same authors showed evidence that quinolones acted as chelating agents vis-à-vis outer membrane-bound divalent cations, notably Mg^{2+} which is well known to antagonize quinolone antibacterial activity (Auckenthaler, Michéa-Hamzhepour & Pechère, 1986). This chelating effect leads to disorganization of this bacterial surface layer, facilitating the further entry of quinolone molecules through the outer membrane in a 'self-promoted' pathway similar to that described for the aminoglycosides (Hancock, Raffle & Nicas, 1981). An alternative route for penetration through the outer membrane is through the water filled protein porin channels. It is likely that for *Esch. coli* quinolone molecules may enter either way, the primary route depending on their solubility: the more hydrophilic compounds via the porin channels and hydrophobic compounds via the 'self-promoted' pathway.

Paradoxical quinolone action on phage replication

Additional insight into the mode of action of quinolones is given by experiments performed with phage T7 which does not seem to require DNA gyrase for growth in *Esch. coli*, since a normal phage burst size occurs at an elevated temperature in host bacteria containing a thermosensitive DNA gyrase. Strikingly, virus replication leading to the same burst was preserved in the presence of nalidixic acid at the elevated, but not at the permissive temperature. Hence inhibition of phage T7 was thought to be due to formation of an irreversible quinolone-gyrase complex, which did not simply have a passive role in blocking a cascade of biochemical reactions, but was thought to act as a poison, mediating cell death (Kreuzer & Cozzarelli, 1979).

Filamentation

Filamentation, caused by the inhibition of cell division, occurs before cell death and may result, at least in part, from antagonism of DNA synthesis and induction of the SOS response (Phillips *et al.*, 1987). Filamentation might also be involved in the killing action of quinolones, since a mutant of *Esch. coli*, incapable of filamenting, appeared to be less susceptible to quinolones than was the parent strain (Walters *et al.*, 1989). Cell enlargement and cessation of septation were also observed in *Staphylococcus aureus* after norfloxacin treatment in a fashion very similar to the alterations produced by cephalexin, which binds almost exclusively to PBP3 (Georgopapadaku, Dix & Mauriz, 1986). Since a significant increase of PBP synthesis has been shown in filaments obtained from nalidixic acid-treated *Esch. coli* cells (Amaral, Schwarz & Lorian, 1986), a direct interaction between quinolone agents and PBPs (or PBP metabolism) represents a hypothesis deserving further investigation.

Unexpected oxacillin-quinolone synergism against methicillin-resistant staphylococci

According to fractional inhibitory concentration indices and killing kinetics, the combination of a quinolone with oxacillin was regularly synergistic or additive against

methicillin-resistant staphylococci (Rohner *et al.*, 1989). Little or no interaction occurred when the same combinations were tested against methicillin-susceptible strains. At first sight, the synergism was somewhat surprising, since the target of oxacillin, that is the bacterial cell wall, appears quite independent from the target of quinolones. Explanatory hypotheses include direct or indirect interactions of quinolones with the PBPs (especially PBP2a which accounts for methicillin resistance) and quinolone induced plasmid cures, since PBP2a regulator genes are known to be located on plasmids. Even though they are little understood, these observations provide *in vitro* evidence for the potential clinical use of quinolones in combination with a β -lactamase resistant penicillin in treating infections due to methicillin-resistant staphylococci.

Quinolones and plasmids

In vitro, quinolones were found to eliminate plasmids of *Esch. coli* at subinhibitory concentrations (Weisser & Wiedemann, 1986). This effect resulted from inhibition of plasmid replication, which seemed to be more sensitive to the quinolone action than chromosomal replication. Curing of plasmids by quinolones remains incomplete and limited however; it is unlikely to be of clinical relevance in the individual patient, but may have ecological implications when quinolones are widely used (Platt & Black, 1987). Furthermore, an *Esch. coli* mating system showed that various quinolones were able to reduce by 90% the efficiency of plasmid conjugation when concentrations were one to six times the MIC for the parent strain (Weisser & Wiedemann, 1987). Again, the phenomenon is probably not clinically important, since the lethal effect of the drug was predominant over the potential inhibition of plasmid transfer. Certainly more pertinent for the clinician is the remarkable scarcity of plasmid-mediated quinolone resistance. As a rare example, a transferable resistance to nalidixic acid has been shown in *Shigella dysenteriae* and *Esch. coli* (Munshi *et al.*, 1987).

Subinhibitory concentrations of quinolones can alter bacterial virulence

The strength of the attractive and repulsive forces involved in bacterial surface interactions with nearby cells are of prime importance for bacterial attachment to host cells, which is a key step in bacterial pathogenesis. Antibiotics that alter the bacterial surface can alter bacterial adhesion (Schifferli & Beachey, 1988) and subsequently phagocytosis. Quinolones do produce bacterial surface changes and, not surprisingly, interfere with the adherence process. Subinhibitory concentrations of pefloxacin ($\frac{1}{2}$ to $\frac{1}{4}$ the MIC) markedly diminished the capacity of *Staph. aureus* for adhesion to buccal cells (Desnottes *et al.*, 1987). Whether these effects involve DNA gyrase or represent another drug action remains as yet unclear, but they may have a clinical impact. In a dog model of arterial grafts, subinhibitory concentrations of pefloxacin were efficient in protecting the foreign body material from *Staph. aureus* infection after bacteraemic challenge (J.-C. Pechère, unpublished observation). However, sub-MICs of quinolones (pefloxacin was not investigated) had no effect on the adherence of slime-producing *Staph. epidermidis* to vascular prostheses in an *in vitro* system (Schmitt *et al.*, 1989), a discrepancy probably due to the absence of host factors in the latter experiments. Other investigations showed that subinhibitory concentrations of pefloxacin decreased the adhesion of *Esch. coli* to human uroepithelial cells, and that this effect was associated with a decrease in P-fimbria formation (Desnottes, Le Roy & Diallo, 1988). In good

agreement with this, quinolones were also capable of altering the affinity of various Enterobacteriaceae for the ileal mucosal cell surface *in vitro* (Edmiston & Goheen, 1989). Subinhibitory concentrations of ciprofloxacin also reduced *Pseudomonas aeruginosa* tissue injury in a rat lung model. This effect was attributed to a decrease in elastase and exoenzyme S production in one non-mucoid isolate, but remained unexplained in a mucoid isolate (Grimwood *et al.*, 1989).

The post-antibiotic effect (PAE)

PAE refers to a temporally limited suppression of regrowth of organisms that have been exposed to an antimicrobial agent for a certain period.

Quinolones have been shown to produce a prolonged (4 h or more) PAE, in both Gram-positive and Gram-negative isolates (Neu *et al.*, 1987). Of particular interest, it seems that the effect can be demonstrated after exposure to quinolones at an acidic pH as well as at high Mg^{2+} concentrations, that is, in conditions that are unfavourable to quinolone antibacterial action. Organisms resistant to quinolones also showed PAE after treatment with quinolone concentrations that are achievable in urine (Neu *et al.*, 1987).

Recently documented antibacterial activity of quinolones

The wide antibacterial spectrum of the new generation of quinolones is now well established (Bellido & Pechère, 1989; Wolfson & Hooper, 1989), but some of the recently documented antibacterial activities may broaden present clinical applications. Several newer compounds have shown enhanced potency against Gram-positive pathogens: temafloxacin (Nye *et al.*, 1989), sparfloxacin (Kojima, Inoue & Mitsuhashi, 1989), WIN 57273 (Jones & Barry, 1990) and Am-1091 (Neu, Novelli & Chin, 1989), to quote only a few. These improvements would be of great interest since infections with *Streptococcus pneumoniae*, *Listeria monocytogenes* and even *Enterococcus faecalis*, which have hardly been amenable to quinolone therapy until now, would be in reach in the future. This, however, will require clinical confirmation. The genus *Nocardia* is another example of a pathogen which shows limited susceptibility to the presently marketed quinolones but is more effectively inhibited by a newer compound, tosufloxacin (Yazawa, Mikami & Uno, 1989).

Several recent studies (Leysen, Haemers & Pattyn, 1989) have confirmed a promising antimycobacterial activity. Quinolones penetrate readily into host cells, a relevant property for treating tuberculosis and leprosy. Hence selected quinolone agents are likely to increase our therapeutic weaponry against these infections. Of the commercially available agents, ofloxacin and ciprofloxacin appear most active, but investigational drugs such as sparfloxacin, and WIN 57273, showed even greater potency. Susceptible mycobacterial species include *Mycobacterium tuberculosis* (Fenlon & Cynamon, 1986; Gorzynski, Gutman & Allan, 1989), *M. paratuberculosis* (Chiadini, 1990), *M. leprae* (Franzblau, 1989; Ramasesh, Krahenbuhl & Hastings, 1989; Franzblau & White, 1990), *M. avium* complex (Khardori *et al.*, 1989) and rapidly growing mycobacterial species (Wallace *et al.*, 1990). In-vitro synergistic activity between ethambutol and fluorinated quinolones was observed against *M. avium* complex, probably associated with an enhanced penetration of the quinolone induced by ethambutol (Hoffner *et al.*, 1989). Clinical experience is as yet limited. Ofloxacin (Tsukamura, Mizuno & Toyama, 1986) and pefloxacin (Fu *et al.*, 1987) have been used

with success in combined therapy for treating tuberculosis. Ofloxacin resistance emerged in cases in which ofloxacin was the only active drug administered (Tsukamura *et al.*, 1985). Other intracellular pathogens against which quinolones have gained recent attention are the *Legionella* species, which showed a good response in an animal model (Saito *et al.*, 1986), and *Coxiella burnetii* (Raoult, Yeaman & Baca, 1989; Yeaman, Roman & Baca, 1989) which was very susceptible to ofloxacin and pefloxacin.

Conclusions

The first fluoroquinolone, pefloxacin, was developed less than ten years ago. During the last decade we have witnessed a rapid increase in information regarding this class of antimicrobial agents, due to the development of new compounds, broadening clinical indications, and increasing knowledge of molecular aspects. Obviously, the field remains very active, and we shall have to follow developments in the years to come.

Acknowledgements

This work was supported by the Fonds National Suisse de la Recherche Scientifique (Grant 31-28007.89).

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