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Mini-review

Antibacterial action of quinolones: From target to network

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ABSTRACT

Quinolones are widely used broad-spectrum antibacterials with incomplete elucidated mechanism of action. Here, molecular basis for the antibacterial action of quinolones, from target to network, is fully discussed and updated. Quinolones trap DNA gyrase or topoisomerase IV to form reversible drug-enzyme-DNA cleavage complexes, resulting in bacteriostasis. Cell death arises from chromosome fragmentation in protein synthesis-dependent or -independent pathways according to distinguished quinolone structures. In the former pathway, irreversible oxidative DNA damage caused by reactive oxygen species kills bacteria eventually. Toxin–antitoxin *mazEF* is triggered as an additional lethal action. Bacteria survive and develop resistance by SOS and other stress responses. Enlarged knowledges of quinolone actions and bacterial response will provide new targets for drug design and approaches to prevent bacterial resistance.

1. Introduction

Quinolones (QLs) are synthetic antimicrobials based on the 4-oxo-1,4-dihydroquinolone skeleton (Fig. 1). The first-generation of QLs, such as nalidixic and oxolinic acids, acts against Gram-negative bacteria and is used to treat urinary tract infections. The second-generation of QLs, such as norfloxacin and ciprofloxacin, is introduced a fluorine atom at position 6 and a bulky piperidine at position 7, broadening the antimicrobial spectrum to *Pseudomonas* species and some Gram-positive organisms, e.g. *Staphylococcus aureus*. The third generation of QLs, with substitutions at the 7 as well as at the 8 position, enhances the activity against Gram-positive bacteria. For instance, levofloxacin and moxifloxacin are active against *Streptococcus pneumoniae* and *S. aureus* that are pathogens responsible for respiratory tract infections, acute otitis and meningitis [1]. Moreover, moxifloxacin is active against *Mycobacterium tuberculosis* which lacks topoisomerase IV (topo IV) [2]. The fourth-generation of QLs is developed with enhanced potency and a broader spectrum including anaerobic bacteria. Gemifloxacin is currently one of the most potent fluoroquinolones against community-acquired pneumonia and acute bronchitis [3]. Trovafloxacin is used against intra-abdominal and pelvic infections [4].

Now, it is quite clear that QLs interfere with chromosomal topology by targeting bacterial type IIA topoisomerases, DNA gyrase and topo IV, trapping these enzymes at the DNA cleavage stage and preventing strand rejoining. As a result, the DNA replication machinery becomes arrested at the blocked replication forks, leading to inhibition of DNA synthesis that immediately causes bacteriostasis [5]. Till now, several crystal structures have been resolved to exhibit accurate structures of the drug-enzyme-DNA ternary complexes, but data gained from these crystal structures demonstrate some contradictions that need to be explained and unified. QL-induced cell death is associated with the formation of double-stranded DNA breaks (DSBs), resulting in chromosome fragmentation and surge of reactive oxygen species (ROS) [5,6]. QLs differ among various derivatives in rate and extent of killing, in the effect of protein synthesis inhibitors on QL lethality and in the need for aerobic metabolism to kill cells [7]. However, the molecular mechanisms of these differences have not been clearly elucidated. It is also known that chromosome-encoded toxin MazF which causes programmed cell death (PCD) is involved in efficient QL killing [8]. To fight against the drug action, SOS regulon and other bacterial response networks are triggered in responses to QLs, providing strategies for bacterial survival and development of resistance.

In this review, a diverse body of knowledge was drawn into the mode of action of QLs from target to network levels. This mainly includes the following details. First, targeting of QLs on DNA gyrase or topo IV resulting in bacteriostatic actions was briefly described

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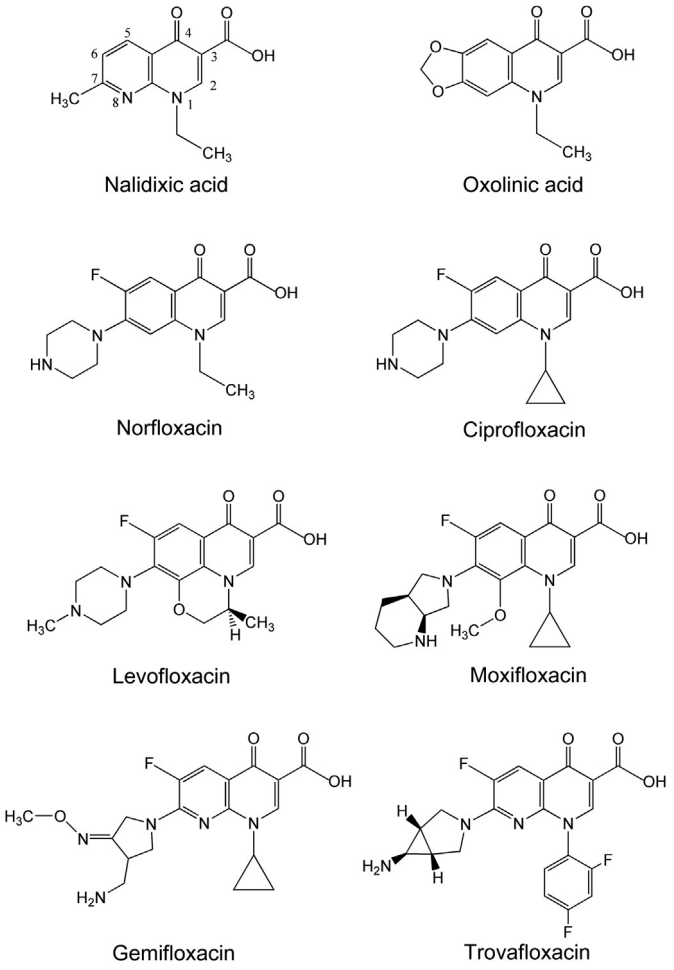

Fig. 1. The chemical structures of QLs.

and crystal structures of drug–enzyme–DNA ternary complexes were updated. Then, different chromosome fragmentation pathways and subsequent events leading to cell death were depicted in details. Last but not least, the bacterial responses induced by QL treatment were summarized. Understanding the mechanisms underlying QL actions and the corresponding bacterial responses could provide new targets for drug design, enhance drug efficiency and prevent bacterial resistance.

2. Primary targets of QLs: DNA gyrase and topoisomerase IV

The first QL, nalidixic acid, was discovered with antimicrobial properties in the by-product distillates in the manufacture of the anti-malarial chloroquine [9], then ten times more potent QL, oxolinic acid, was synthesized five years later [10]. Goss et al. [11,12] first showed that nalidixic acid was a selective, immediate and reversible inhibitor of bacterial DNA synthesis. Soon after the discovery of bacterial gyrase in 1976 [13], both Gellert and Cozzarelli [14,15] demonstrated that the supercoiling activity of the purified gyrase, extracted from wild-type cells but not from resistant *nalA* mutants, was inhibited by nalidixic acid and oxolinic acids. In 1978, Higgins et al. [16] confirmed that subunit A of DNA gyrase (GyrA) was the product of the gene controlling sensitivity to nalidixic acid. These results suggest that DNA gyrase is the primary target of QLs.

Gyrase, belonging to type IIA topoisomerases, functions as a heterotetramer composed of two GyrA subunits and two GyrB subunits [17]. Gyrase's enzymatic activity is essential for the regulation of DNA superhelicity, bacterial replication, transcription initiation and elongation. Gyrase generates a pair of single-stranded breaks (SSBs) in the region of DNA wrapped around the enzyme. One DNA gate is opened through which another stretch of DNA can pass. After that, the gate closes and hydrolysis of ATP resets gyrase for another round.

In 1990, Kato et al. [18] discovered a homolog of gyrase called topoisomerase IV (topo IV). Like gyrase, topo IV is composed of two ParC subunits and two ParE subunits [19]. Soon after the discovery of topo IV, it became clear that gyrase was not the only intracellular target of QLs [20,21]. In *Escherichia coli*, mutations mapped in *parE* [22] or near *parC* [23] were identified conferring a high level of resistance. Since QL resistance alleles in *parE* or *parC* did not confer resistance by themselves, topo IV must be a secondary target in *E. coli* [22–25]. This is also the same case for *Neisseria gonorrhoeae* [26].

However, things are rather different for some Gram-positive strains. Clinical isolates of *S. aureus* that were resistant to a moderate level of ciprofloxacin contained a mutation in *parC* (*grlA*) while isolates that were resistant to a high concentration of ciprofloxacin exhibited an additional mutation in *gyrA* [27]. Purified topo IV was inhibited by QLs from a variety of assays, including measurement of DNA cleavage, decatenation and relaxation activity [25,28,29]. Gyrase and topo IV from both *S. aureus* and *E. coli* were purified to study the fluoroquinolone sensitivity [30]. The supercoiling activity of *S. aureus* gyrase was at least 500-fold less sensitive to ciprofloxacin than that of *E. coli* gyrase and about 6-fold less sensitive than the decatenating activity of *S. aureus* topo IV. These observations suggest that topo IV, rather than gyrase, is the primary target of ciprofloxacin in *S. aureus*. The same phenomenon occurs in *S. pneumoniae* [31,32].

In summary, it is now quite clear that bacteria contain two topoisomerase targets for QLs. In Gram-negative bacteria the primary target is gyrase, while in Gram-positive bacteria the primary target is generally topo IV. Since the two enzymes have different functions, it is likely that bacteria differ in their response to QLs according to which enzyme is the primary target.

3. Bacteriostatic actions of QLs

3.1. Formation of drug-enzyme-DNA complex

Formation of a QL–enzyme–DNA complex that contains broken DNA is the central event in the interaction between the QLs and gyrase or topo IV. Footprinting experiments suggested that the QLs allowed gyrase to proceed to a conformational change in which additional DNA was wrapped around gyrase [33]. The QL–enzyme–DNA complex formation is readily reversible. Chromosome fragmentation was eliminated after the drug removed or by mild heat treatment (60 °C) [34]. Addition of a protein denaturant such as sodium dodecyl sulfate (SDS) to the ternary complexes would release DNA ends [14,15,29,34]. Nevertheless, the drugs cannot cause generation of free DNA ends inside bacterial cells since intact nucleoids containing negatively supercoiled DNA could be isolated from oxolinic acid treated bacteria in a cell lysis procedure without SDS [34,35].

Comparison of the nucleotide sequences at the cleavage sites reveals a loose consensus sequence [36–39]. Not all of the cleavage sites identified on a given DNA molecule were cleaved when oxolinic acid is added to cells [34]. Cleavage is especially frequent at a small number of specific sites called toposites on the chromosome [40]. A large number of weaker sites also exist [41]. It is speculated that the small number of strong interaction sites are used by gyrase to maintain superhelical tension in the chromosome as a whole and the weak and widely dispersed sites allow gyrase to provide local swiveling needed for transcription and replication.