

Quinolone Resistance: More Than Topoisomerases

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INTRODUCTION

Resistance mechanisms to various antibiotics are known as follows: 1, alteration of target sites; 2, existence of degrading enzymes; 3, modification of antibiotics; 4, reduced permeability; 5, existence of efflux pump. Among these, quinolone resistance is a result from the modification of target sites and reduced intracellular concentration due to the reduced permeability and efflux pump. In this talk, I will show you how reduced permeability and efflux pump as well as mutations in the target sites of quinolone-DNA gyrase and topoisomerase IV can cause quinolone resistance.

HOW QUINOLONE INHIBITS DNA GYRASE

MUTATIONS IN TARGET SITES: DNA GYRASE AND TOPOISOMERASE IV

Target sites of quinolones are DNA gyrase and topoisomerase IV ^{4, 8, 13, 24, 28, 39}. DNA gyrase is responsible for DNA supercoiling and topoisomerase IV for cell partitioning after DNA replication. DNA gyrase is composed of two GyrA and two GyrB subunits and topoisomerase IV is composed of two ParC and two ParE subunits and both enzymes have a great homology ²⁶. GyrA and ParC cut DNA strand and GyrB and ParE provide energy for this reaction. Tyr122 of GyrA subunit of DNA gyrase binds to one DNA strand. Then phosphodiester bond becomes cleaved, the other strand passes through the active site releasing the superhelicity once, and then DNA gyrase ligates two cleaved strands. When quinolone is present, quinolone, DNA, and DNA gyrase form a ternary complex ¹⁰. Quinolone inhibits DNA releasing from DNA gyrase and prevents religation of DNA strands producing DNA breakage ^{1, 9}. When a mutation occurs around Tyr122, DNA gyrase cannot bind to quinolone and form a quinolone-DNA-DNA gyrase ternary complex. This is how a mutation in QRDR renders resistance. As shown in Tables, many quinolone resistance mutations have been reported and most of them are located in the quinolone resistance determining region (QRDR) in GyrA subunit of DNA gyrase and ParC subunit of topoisomerase IV. Nowadays, several regulatory genes such as *marA*, *soxS*, and *robA* have been found to be responsible for quinolone resistance.

REDUCED INTRACELLULAR CONCENTRATION : PERMEABILITY AND EFFLUX

Gram-positive and-negative cells have different cell structures. Gram-positive cells have one thick peptidoglycan layer while gram-negative cells have inner and outer membranes and periplasmic space between two membranes. Hydrophilic materials such as amino acid need special gates to get into cells. These gates are outer membrane proteins, called as Omp (outer membrane protein) or porin. Hydrophilic quinolones must be transported through these proteins, too³⁷). When these gate proteins are not expressed, intracellular quinolone concentration decreases and MIC increases. In case of hydrophobic quinolone which is uptaken through the phospholipid layer, change in phospholipid composition can increase MICs²²). Outer membrane proteins can be easily detected by SDS-denaturing gel electrophoresis after outer membranes were isolated. Phospholipid composition can be detected by gas chromatography or urea-SDS-denaturing gel electrophoresis¹⁷).

QUINOLONE EFFLUX PUMP

Another reason for the reduced intracellular quinolone concentration is the existence of efflux system. Efflux systems have been reported in various bacteria including *E. coli*, *Pseudo. aeruginosa*, *Staph. aureus*, and *Strep. pneumoniae*. Unlike efflux pumps in eukaryotes, efflux pumps in bacteria use the proton motive force as an energy source. When CCCP, which dissipates the proton motive force, is added to cells, we can easily observe the increase in the intracellular quinolone concentration as shown in Figure 1. Even though the existence of efflux pump cause only a 2 to 4 fold increase in MICs, it becomes important for treating the borderline resistant bacteria. *NorA* in *Staph. aureus* was the first reported gene for efflux pump²⁰). In addition to this, several other genes such as *opr*, *mex*, and *nfx* etc. have been found. It looks like that every bacterium has efflux pumps intrinsically. When these efflux proteins were expressed in large amount, then the resistance occurs as a result. As Kohler et al.¹⁵) and I¹⁸) suggested that efflux system have a specificity so each quinolone is effluxed via specific efflux system as shown in Table 5.

IRON AFFECTS THE INTRACELLULAR OFLOXAICN CONCENTRATION

Recently I found that iron could increase the susceptibilities of ofloxacin-resistant but not ciprofloxacin- or norfloxacin-resistant *Pseudo. aeruginosa*. When iron was added to cells, intracellular ofloxacin concentrations increased even though the efflux system was inhibited by the addition of CCCP (Figure 2). Since Poole's group suggested TonB-related efflux system³⁹), I added ouabain to inhibit ATPase for Ton-B system. Still iron increased intracellular ofloxacin concentration as shown in Figure 3. Results suggested that iron decreased MICs of ofloxacin by increasing uptake. So, there must be another ofloxacin import system activated by iron besides porin F and

this system needs outer membrane proteins as shown in experiments done with salicylate- and succinate minimal medium grown cells.

SUGGESTION TO OVERCOME AND PREVENT RESISTANCE

As everybody agrees that the occurrence of quinolone resistant bacteria as well as other antibiotics resistant bacteria should be prevented and must be treated as soon as possible. This is not an easy task and can be accomplished only through cooperation among people in microbiology, biochemistry, pharmaceutical industry, human medicine and veterinary medicine. Microbiologist and biochemist have to study the resistant mechanism, provide information how to overcome resistance, study the metabolism to find new target sites, and do structure and relationship study (SAR) to be used as a guideline for developing a new antibiotics. People in pharmaceutical industry should develop new antibiotic with little chance to cause resistance or modify old antibiotics to prevent resistance. Doctors should identify the causative agent and use narrow spectrum antibiotics. Also they have a responsibility to educate patients to follow their instruction to avoid developing resistant bacteria. Since there is a great possibility that the resistance can be transferred to human bacteria from animal bacteria and quinolone is one of the major antibiotics used in animal industry, we should closely watch the occurrence of resistant bacteria and restrict the use of antibiotics in animal only for therapeutic purpose not used as feed additives.

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Table 1. Target site mutations found in *Escherichia coli*.

DNA gyrase		Topoisomerase IV	
GyrA	GyrB	ParC	ParE
Ala67 ; æ Ser	Ser426 ; æ Asn	Gly78 ; æ Asp	Leu445 ; æ His
Ser80 ; æ Arg	Lys447 ; æ Glu	Gly78 ; æ Cys	
Gly81 ; æ Asp		Ser80 ; æ Ile	
Gly81 ; æ Cys		Glu84 ; æ Gly	
Asp82 ; æ Gly		Glu84 ; Lys	
Ser83 ; æ Ala			
Ser83 ; æ Leu			
Ser83 ; æ Trp			
Ala84 ; æ Pro			
Asp87 ; æ Asn			
Asp87 ; æ Gly			
Gln106 ; æ Arg			
Gln106 ; æ His			

Ref) 2, 3, 6, 7, 8, 21, 23, 26, 28, 30, 33

Table 2. Target site mutations found in *Pseudomonas aeruginosa*

DNA gyrase		Topoisomerase IV	
GyrA	GyrB	ParC	ParE
Thr83 ; Ile			
Asp87 ; æ Asn			
Asp87 ; æ Gly			
Asp87 ; æ Tyr			
Asn116 ; æ Tyr			

Ref) 6, 11, 14, 15, 22, 27

Table 3. Target site mutations found in *Staphylococcus aureus*.

DNA gyrase		Topoisomerase IV	
GyrA	GyrB	GrlA	GrlB
Asp73 ; æ Gly	Asp437 ; æ Asn	Ser80 ; æPhe	Asp432 ; æAsn
Ser84 ; æ Ala	Arg458 ; æ Gln	Ser80 ; æTyr	Asn470 ; æAsp
Ser84 ; æ Leu		Ser81 ; æPro	
Ser85 ; æ Pro		Glu84 ; æLys	
Glu88 ; æ Lys		Ala116 ; æGlu	
Asp82 ; æ Gly		Ala116 ; æPro	

Ref) 4, 5, 10, 17, 24, 29, 31

Table 4. Target site mutations found in *Streptococcus pneumoniae*.

DNA gyrase		Topoisomerase IV	
GyrA	GyrB	ParC	ParE
Ser81 ; æ Phe	Asp435 ; æ Asn	Ser79 ; æPhe	Asp435 ; æAsn
Ser83 ; æ Phe	Glu474 ; æ Lys	Ser79 ; æTyr	Pro454 ; æ Ser
Ser83 ; æ Tyr		Ser80 ; æPro	
Ser84 ; æ Phe		Asp83 ; æAla	
Ser84 ; æ Tyr		Asp83 ; æGly	
Glu85 ; æ Lys		Asp83 ; æHis	
Glu87 ; æ Gln		Asp83 ; æTyr	
Glu87 ; æ Lys		Ala84 ; æ Thr	
Trp93 ; æ Arg		Arg95 ; æ Lys	
		Lys137 ; æ Asn	

Ref) 13, 16, 19, 25

Table 5. Ciprofloxacin and ofloxacin concentrations inside cells^a

CCCP	Quinolone	Susceptible strain	Resistant strain (PA150)	Resistant strain (PA300)
		%	%	%
- ^b	Ofloxacin	42	40	59
	Ciprofloxacin	58	60	41
	total quinolones	100	100	100
+ ^c	Ofloxacin	42	42	33
	Ciprofloxacin	58	58	66
	total quinolones	100	100	100

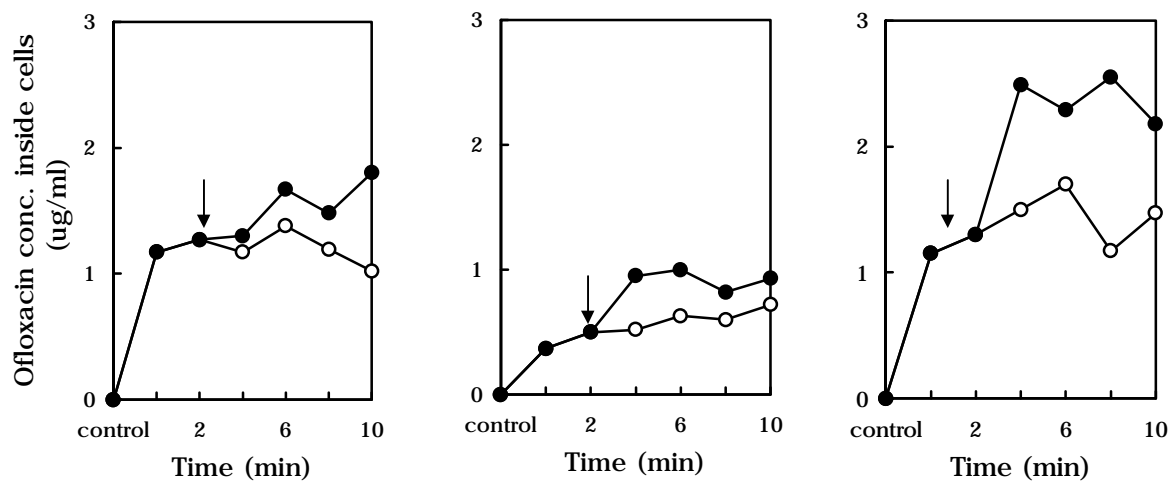
^a Cells were incubated with 50 μ g/ml of ciprofloxacin and 50 μ g/ml of ofloxacin.

Quinolones were extracted from cells with boiling, separated using a C18 column, and detected at 254 nm.

^b Cells were incubated with quinolones in the absence of CCCP.

^c Cells were incubated with quinolones in the presence of CCCP.

Figure 1. Intracellular quinolone concentration in the presence of CCCP.



P. aeruginosa

E. coli

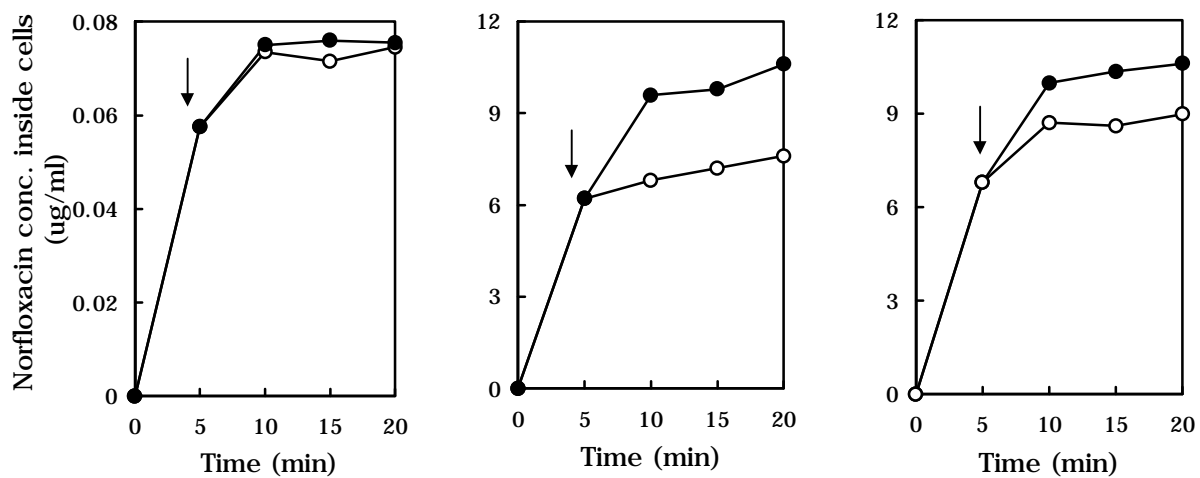


Figure 2. Effect of iron on the intracellular ofloxacin concentration.

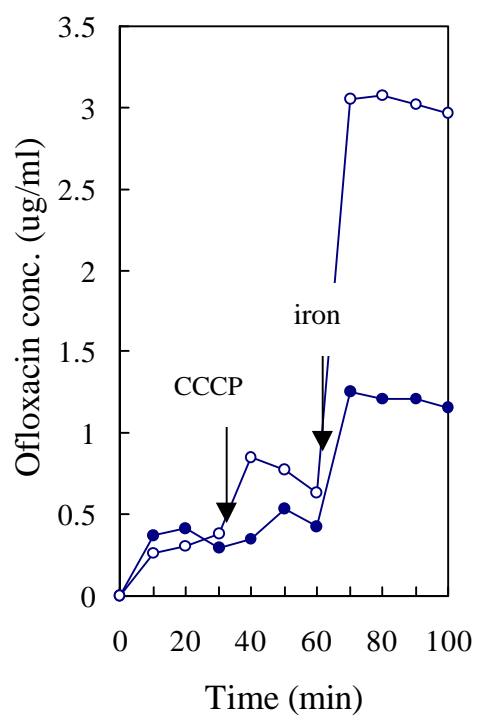


Figure 3. Effect of iron on cells treated with CCCP and ouabain.

