Inhibitors of Bacterial Topoisomerases: Mechanisms of Action and Resistance and Clinical Aspects

Peter Heisig
Pharmazeutische Biologie Universität Hamburg, Hamburg, Germany

Received: May 26, 2000; Accepted: August 19, 2000

Abstract: The quinolone class of inhibitors of bacterial type II topoisomerases has gained major clinical importance during the last years due to improvements in both pharmacokinetic and pharmacodynamic properties. These include favorable bioavailability allowing oral administration, good tolerability, high tissue concentrations as well as superior bactericidal activity against a broad spectrum of clinically relevant pathogens, like enterobacteria, Pseudomonas aeruginosa, Staphylococcus aureus, and Streptococcus pneumoniae. In addition, no enzymatic mechanism of drug inactivation exists in bacteria and no indications for transfer of clinically relevant resistance exist. Nevertheless, resistance is being increasingly reported, even for naturally highly susceptible species like Escherichia coli. The underlying mechanisms of resistance include alterations in both bacterial targets. DNA gyrase and topoisomerase IV, often combined with mutations affecting drug accumulation, e.g., by increased drug efflux, reduced drug influx, or both. Investigations aiming at understanding the molecular mechanisms of quinolone action and resistance in more detail should provide a basis for a rational design of more potent derivatives. In addition, a prudent use of these highly valuable “magic bullets” is necessary to preserve their potential for the future.

Key words: Bacterial topoisomerase, fluoroquinolone, bacteria and antibiotic resistance.

Introduction

According to the World Health Report on Infectious Diseases 2000 overcoming antibiotic resistance is the major issue of the WHO for the next millennium. Currently, antibiotics provide the main basis for a causative therapy of bacterial infections. However, the high genetic variability of bacteria enables them to rapidly evade the action of antibiotics by developing antibiotic resistance. Thus, there has been a continuing search for new, more potent antibiotics.

During the last decades a limited number of well established classes of antibiotics, e.g., β-lactams, macrolides or quinolones, have been investigated extensively to identify the mechanism of action and the molecular structure of the target site. Based upon this knowledge, new antibiotics were designed by chemical modification of approved prototype compounds. However, this led to a limitation of therapeutic options for those bacteria which have already developed high resistance to these prototypes.

In contrast, the novel genomics approach uses DNA sequence data bases to identify novel lethal targets. However, sophisticated systems for high-throughput screening of chemical and biological molecule libraries are needed in order to identify lead compounds, which subsequently require additional research capacities for elucidating the underlying mechanism of action (1).

Starting with a well characterized target structure to screen for alternate, chemically unrelated inhibitors seems to be another promising approach. Thus, bacterial type II topoisomerases can be considered as targets not only for known but also for potential novel antibiotics.

DNA Topoisomerases

Topoisomerases play an essential role for the control of the three-dimensional DNA structure in all cells (eubacteria, eukaryote, archaea). According to their mechanism of action topoisomerases are classified as type I or type II enzymes [for a recent review see (2)].

The first topoisomerase detected was the bacterial type I enzyme, topoisomerase I (3), which in cooperation with DNA gyrase, a bacterial type II enzyme, enzymatically controls the maintenance of the DNA supercoiling degree (4). Among all topoisomerases DNA gyrase is unique by its ability to introduce so-called negative supercoils into covalently closed circular (ccc) double stranded DNA in the relaxed state (Fig. 1).

DNA gyrase holoenzyme consists of two pairs of subunits, GyrA and GyrB, the products of the corresponding genes gyrA and gyrB, respectively, and forms a tetrameric structure (A2B2) (Fig. 2). Both subunits contain functional domains involved in interactions with DNA, with ATP (ATP-binding motif in GyrB), and between subunits A and B (for a review see ref. (5)) Fig. 2.

---

1 Experimental work was performed at the Department of Pharmaceutical Microbiology at the University of Bonn

© Georg Thieme Verlag Stuttgart · New York
ISSN: 0032-0943
The current model explaining the molecular mechanism of action of type II topoisomerases is based upon biochemical and genetic investigations of DNA gyrase from *E. coli* and crystallographic analysis of a homologous type II topoisomerase from *Saccharomyces cerevisiae* (6). (7). This model postulates a multi-step mechanism schematically outlined in Fig. 2.

Recently, another bacterial type II topoisomerase, topoisomerase IV (top IV), has been identified in *E. coli* (8). Both enzymes – gyrase and top IV – share several structural and functional features. Based upon protein sequence homology to DNA gyrase, analogous domains (e.g., ATP-binding) are assigned to subunits A (ParC) and B (ParE) of top IV. In contrast to DNA gyrase, top IV catalyzes the separation (decatenation) of two double-stranded ccc DNA molecules intertwined like links of a chain (Fig. 1). This is a prerequisite for the termination of the DNA replication and the subsequent cell division (9).

While the gyrase-mediated reaction involves intramolecular strand passage (the T-segment belongs to the same DNA molecule as the G-segment; Fig. 2) top IV favors an intermolecular strand transfer (T-segment is part of a second ccc DNA molecule). The ubiquitous distribution among all bacteria and the uniqueness of the supercoiling reaction makes DNA gyrase an “ideal” lethal target for broad-spectrum antibiotics.

### Inhibitors of Bacterial Type II Topoisomerases

Several inhibitors of DNA gyrase and/or top IV have been identified so far. These include (i) the GyraA-targeting 3.2 kD glycine-rich peptide MccB17, which has been isolated from enterobacteria carrying the corresponding biosynthetic gene cluster on a plasmid (10), (ii) the cyclic peptide cyclohexalidine isolated from *Streptomyces* and its derivative GR122222X (Fig. 3), which interfere with binding of ATP to the Gyrb subunit (11), (iii) cinodine (Fig. 3), a glyccinnamoylspermidine antibiotic, which is produced by *Nocardia* species (12) and targets gyrase as indicated by studies with cinodine-resistant mutants of *E. coli* (13), (iv) clorocidin (Fig. 3), a terpenoid antibiotic from *Fusidium viride*, which besides its cytotoxic activity on mammalian topoisomerase II also acts on the DNA gyrase A-subunit (14), (15) (Fig. 3).

Since top IV has only recently been identified as a second quinolone target, most of these drugs have not yet been investigated for their activity on this enzyme. There is one report on the inhibitory activity of the glycosylated flavonoids rutin and isoquercitrin preferentially on top IV (16).

None of these compounds has yet been developed for clinical use as antimicrobials, probably due to high toxicity (activity on eukaryotic targets), unfavourable pharmacokinetic properties (high molecular weight causing low membrane permeability) or risk of cross-resistance (overlapping binding sites).
In contrast, two classes of inhibitors of bacterial type II topoisomerases, coumarin antibiotics and quinolones, are successfully used for treatment of infections due to Gram-positive and Gram-negative pathogens.

The coumarin antibiotics novobiocin (Fig. 3), chlorobiocin, and coumermycin A₅, which are naturally produced by Streptomyces species, interact with the N-terminal amino acids of the CytB subunit thereby stabilizing an enzyme conformation of low affinity for ATP (17), (18), (19). Due to their high molecular masses, coumarin antibiotics do not sufficiently penetrate the outer membrane of most Gram-negative bacteria (20). Thus, they are of minor clinical importance and are rarely used for the treatment of infections caused by some Gram-positive pathogens.

Quinolones are synthetic compounds first described in 1962 by Lesher and coworkers. Nalidixic acid (Fig. 4), the prototype of the quinolone class of antimicrobials, actually is an aza-derivative (1,8-naphthyridine) of 4-quinolone-3-carboxylic acid.
Table 1  Quinolone susceptibilities of various bacterial pathogens.

<table>
<thead>
<tr>
<th>Species</th>
<th>Nalidixic acid</th>
<th>Norfloxacin MIC&lt;sub&gt;50&lt;/sub&gt; [μg/ml]</th>
<th>Ciprofloxacin</th>
<th>Clinafloxacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>4</td>
<td>0.125</td>
<td>0.015</td>
<td>0.008</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>&gt;128</td>
<td>4</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>A. baumannii</td>
<td>8</td>
<td>8</td>
<td>0.5</td>
<td>0.125</td>
</tr>
<tr>
<td>H. influenzae</td>
<td>1</td>
<td>0.06</td>
<td>0.015</td>
<td>0.004</td>
</tr>
<tr>
<td>H. pylori</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.5</td>
<td>0.125</td>
</tr>
<tr>
<td>M. pneumoniae</td>
<td>n.d.</td>
<td>16</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>B. fragilis</td>
<td>64</td>
<td>2</td>
<td>0</td>
<td>0.06</td>
</tr>
<tr>
<td>S. aureus</td>
<td>&gt;128</td>
<td>16</td>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td>S. pneumoniae</td>
<td>&gt;128</td>
<td>8</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>E. faecalis</td>
<td>&gt;128</td>
<td>2</td>
<td>1</td>
<td>0.25</td>
</tr>
<tr>
<td>M. tuberculosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* n.d.: not data.

It is derived from a by-product of the chloroquine synthesis (21). Nalidixic acid shows clinically relevant drug concentrations only in urine and has a narrow range of activity against some Gram-negative enterobacteria, restricting its clinical use to the treatment of enterobacterial urinary tract infections. Attempts to broaden its antibacterial spectrum of activity led to norfloxacin, a 4-quinolone-3-carboxylic acid carrying a 6-fluoro and a 7-piperazinyl substituent (Fig. 4, Table 1 (22)). In addition to an at least 100-fold increase in the antibacterial activity against Gram-negative bacteria, norfloxacin has also a weak, though significant activity against Staphylococcus aureus (23). Heterocyclic substituents at the C7-position, e.g., gemifloxacin have improved the activity against Gram-positives (Fig. 4, Table 1), another significant improvement came from variations at positions C1 and C8 leading to sitafloxacin, moxifloxacin or gatifloxacin (Fig. 4).

According to their clinical indication and application, Naber et al. recently suggested to divide fluoroquinolones into four classes (Table 2) (24). While ciprofloxacin is the most active fluoroquinolone against Gram-negative pathogens including Pseudomonas aeruginosa, the new derivatives, e.g., those belonging to classes III and IV are also active against various Gram-positives, like S. aureus, S. pneumoniae (25), (26), (27), (28), (29), (30), as well as Mycobacterium tuberculosis (31), (32), (33) (Table 1). Thus, the new fluoroquinolones have a high efficacy in the treatment of respiratory tract infections covering not only the common pathogens S. pneumoniae, H. influenzae, and Moraxella catarrhalis, but also the uncommon Chlamydia pneumoniae, Legionella pneumophila, and Mycoplasma pneumoniae (34).

Table 2  Classification of fluoroquinolones (24).

<table>
<thead>
<tr>
<th>Class</th>
<th>Application</th>
<th>Drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Oral, exclusively</td>
<td>Norfloxacin, Pefloxacin</td>
</tr>
<tr>
<td>II</td>
<td>Systemic, wide indication</td>
<td>Enoxacin (oral), Fleroxacin, Ofl oxacin, Ciprofloxacin</td>
</tr>
<tr>
<td>III</td>
<td>Increased activity against Gram-positive and &quot;atypical&quot; pathogens (mycobacteria)</td>
<td>Gatifloxacin*</td>
</tr>
<tr>
<td>IV</td>
<td>Like III + anaerobes</td>
<td>RIF, Tetracycline, Moxifloxacin, Clinafloxacin*</td>
</tr>
</tbody>
</table>

* Not yet licensed (withdrawn).

Quinolones act by formation of a stable ternary complex consisting of (i) DNA, covalently attached to (ii) DNA gyrase A subunits of the A,B, tetramer, and (iii) quinolones thereby preventing the relaxation step. As a consequence the progress of DNA- and RNA-polymerases along the DNA is blocked. A stop of DNA replication induces the SOS response (38), which triggers the synthesis of new proteins resulting in arrest of cell division, cessation of the respiratory chain and finally in cell death by an as yet incompletely understood mechanism (39).

Mechanism of Action of 4-Quinolones

Quinolone antibacterials display bactericidal activity against dividing cells (Fig. 5a) resulting in a rapid decrease of the viable cell count by several orders of magnitude (35). This is due to the inhibition of the replicative DNA synthesis rather than protein or RNA synthesis (Fig. 5b) (36). Evidence for DNA gyrase as the target of quinolones came from Gellert et al., who found the inhibitory concentration of oxolinic acid for DNA gyrase isolated from a quinolone-susceptible strain to be much lower than that of its quinolone-resistant derivative carrying a mutation in the structural gene gyrA, formerly called nalA (Fig. 5c) (37).

Mechanisms of Bacterial Resistance to Quinolones

In general, two basic genetic events can lead to antibiotic resistance – acquisition of additional DNA coding for a resist-
Inhibitors of Bacterial Topoisomerases: Mechanisms of Action and Resistance and Clinical Aspects

Planta Med 67 (2001) 7

Figure 5a: Bactericidal activity of quinolones. Addition of nalidixic acid in different concentrations (1, 5, and 25 μg/ml) to exponentially growing cells of a quinolone-susceptible strain of E. coli resulted in a concentration-dependent reduction in the viable cell count during 180 min. These data indicate the bactericidal mechanism of quinolones. [Data according to results of (35)].

Figure 5b: Mechanism of action of quinolones - inhibition of DNA synthesis. The addition of nalidixic acid (final concentration 50 μg/ml, ○○○) did not affect the incorporation of radio-labelled precursors of protein synthesis (³¹C-arginine, center) and of RNA synthesis (³¹C-uracil, right) by cells of E. coli compared to an untreated control (●●●). In contrast, the incorporation of ³¹C-thymidine (left) is abolished immediately after the addition of nalidixic acid indicating that quinolones are inhibitors of DNA replication. [Data according to results of (36)].

Figure 5c: Molecular mechanism of action of quinolones - inhibition of DNA gyrase. The enzymatic supercoiling activity of DNA gyrase (gyrA, white columns) isolated from a quinolone susceptible isolate of E. coli is inhibited by the addition of oxolinic acid in a concentration dependent manner. No effect is observed with DNA gyrase (gyrA, shaded columns) isolated from an E. coli strain carrying a single mutation in the gene gyrA (formerly nalA) coding for a quinolone-resistant A subunit. [Data according to results of (37)].

In contrast to the older, unfluorinated quinolones growth of both types of mutants, nalA and nalB, can be inhibited by new fluorinated quinolones at clinically relevant concentrations. As a consequence, using even high inocula of about 10¹² cells it is impossible to select single-step mutants of E. coli with clinically relevant resistance to fluoroquinolones (42).

The lack of resistance gene transfer and enzymatic drug inactivation together with the high activity of fluoroquinolones against single-step mutants of naturally quinolone-susceptible bacteria, like E. coli, led some investigators to deny the ability of these bacteria to develop clinically relevant resistance towards fluoroquinolones.

Obviously, this optimistic point of view underestimated the high genetic variability of bacteria: During the last decade an increase in the prevalence of fluoroquinolone resistant isolates of E. coli from less than 0.5% to about 10% has occurred (43). Such highly resistant clinical isolates show increases in the MICs of, e.g., ciprofloxacin from 0.015 μg/ml for a susceptible isolate to 64 μg/ml and higher for resistant isolates (44), (45), (46), (47).

Target-Mediated Resistance Mechanisms in Gram-Negatives

To elucidate the molecular basis for high-level fluoroquinolone resistance, sequential mutants of a wild-type quinolone-susceptible isolate (WT) have been selected in vitro. Three se-
Table 3 Impact of GyrA double mutation S83L-D87G on ciprofloxacin (cip) susceptibility of isolated enzyme(3rd and whole cells).

<table>
<thead>
<tr>
<th>Strain</th>
<th>GyrA-mutation</th>
<th>IC50 (μg/ml)</th>
<th>MIC(μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td></td>
<td>1</td>
<td>0.015</td>
</tr>
<tr>
<td>M I</td>
<td>S83L</td>
<td>10</td>
<td>0.5</td>
</tr>
<tr>
<td>M II</td>
<td>D87G</td>
<td>10</td>
<td>0.25</td>
</tr>
<tr>
<td>M III</td>
<td>S83L/D87G</td>
<td>&gt;2000</td>
<td>64*(selected in vitro)</td>
</tr>
<tr>
<td>Wf-J</td>
<td>S83L/D87G</td>
<td>&gt;2000</td>
<td>1*(mutagenized)</td>
</tr>
<tr>
<td>32T</td>
<td>S83L/D87G</td>
<td>&gt;2000</td>
<td>64*(clinical isolate)</td>
</tr>
</tbody>
</table>

* Obtained by in vitro mutagenesis.

Table: Impact of GyrA double mutation S83L-D87G on ciprofloxacin (cip) susceptibility of isolated enzyme(3rd and whole cells).

<table>
<thead>
<tr>
<th>Strain</th>
<th>GyrA-mutation</th>
<th>IC50 (μg/ml)</th>
<th>MIC(μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td></td>
<td>1</td>
<td>0.015</td>
</tr>
<tr>
<td>M I</td>
<td>S83L</td>
<td>10</td>
<td>0.5</td>
</tr>
<tr>
<td>M II</td>
<td>D87G</td>
<td>10</td>
<td>0.25</td>
</tr>
<tr>
<td>M III</td>
<td>S83L/D87G</td>
<td>&gt;2000</td>
<td>64*(selected in vitro)</td>
</tr>
<tr>
<td>Wf-J</td>
<td>S83L/D87G</td>
<td>&gt;2000</td>
<td>1*(mutagenized)</td>
</tr>
<tr>
<td>32T</td>
<td>S83L/D87G</td>
<td>&gt;2000</td>
<td>64*(clinical isolate)</td>
</tr>
</tbody>
</table>

* Obtained by in vitro mutagenesis.

Determining for WT and its derivatives the DNA sequence of the so-called "quinolone resistance-determining region" (QRDR) of gyrA, where all known single step mutations associated with quinolone resistance map (54), revealed a serine-83 to leucine (S83L) mutation in mutant M1 and, in addition, an aspartate-87 to glycine (D87G) mutation in mutant MII (55). DNA supercoiling activity of DNA gyrase isolated from mutant MII is as refractory to ciprofloxacin (IC50 > 1500 μg/ml) as that of DNA gyrase reconstituted from a wild type GyrB subunit and a genetically engineered GyrA subunit containing solely the double mutation S83L-D87G (Table 3). Expanding the DNA sequence analyses to clinical isolates with high-level fluoroquinolone resistance revealed that it is associated with a gyrA double mutation primarily affecting codons for serine-83 and aspartate-87 (53). In order to investigate if this double mutation is sufficient for the expression of high-level fluoroquinolone resistance, the susceptibilities to ciprofloxacin were determined for mutant MII selected in vitro and mutant WT-3 which was obtained by in vitro mutagenesis. WT-3 contains only the gyrA double mutation S83L-D87G in the genetic background of WT. Mutant WT-3 was as susceptible as the single step mutant M1 (Table 3), indicating the presence of an additional, yet unidentified mutation in MII. Data of Hoshino et al. (56) demonstrating that fluoroquinolones inhibit in vitro the activity of topo IV at concentrations several fold higher than those required for inhibition of DNA gyrase, supported the idea that topo IV is an additional target of fluoroquinolones in E. coli.

This hypothesis was challenged by determining the DNA sequence of a fragment from parC containing the region homologous to the QRDR of gyrA for the susceptible parent strain WT and its derivatives M1, MII, and MIII. Compared to WT no alteration was found for M1 and MII, however, mutant MIII did carry a mutation resulting in a serine-80 to isoleucine change. Serine-80 of ParC is homologous to serine-83 for GyrA (Fig. 6).

Fig. 6 Conserved amino acid sequences in GyrA and ParC of various Gram-negative bacteria and quinolone resistance mutations. According to the respective proteins from E. coli amino acids of the quinolone resistance-determining region (QRDR, (54) of subunit A of DNA gyrase (GyrA, top) and the homologous region of subunit B of topoisomerase IV (ParC, bottom) were numbered. Naturally variant amino acids of the respective genes detected in quinolone-susceptible strains of several Gram-negative species are given below the respective QRDR. Mutations associated with quinolone resistance in various Gram-negative isolates in subunits A of both target topoisomerases are shown under below the line the respective amino acid positions (44, 45, 46, 47, 53, 60, 92, 93, 94).

Subsequent DNA sequencing of clinical isolates with high-level fluoroquinolone resistance revealed the presence of at least one mutation within the QRDR-like region of parC. All amino acids in ParC associated with quinolone resistance were homologous to those in GyrA (Fig. 6). Moreover, transferring a plasmid-coded quinolone-susceptible allele of parC obtained from strain WT into parC mutant strains (e.g., MII) resulted in a reduction of the fluoroquinolone resistance to the level of a single gyrA mutant, but not to that of WT. This dominance effect was not observed if the parC gene obtained from mutant MII was transferred (44).

These results indicating the requirement of a combinatorial of mutations affecting both targets of quinolones, topo IV and DNA gyrase, are required for high-level resistance, do not only apply to E. coli (57), (58), (59) but also to other Gram-negative pathogens (60). Strains carrying a combination of three target mutations are resistant even to new derivatives like cinafloxacin, sitafloxacin showing highest antibacterial activity (61).

Target-Mediated Resistance Mechanisms in Gram-Positives

Ciprofloxacin and ofloxacin carrying modified N1-substituents were the first fluoroquinolones to be licensed for a broad indication including infections of the bone, the skin, and the soft tissue, which can be caused by S. aureus, a Gram-positive pathogen. However, the antibacterial activities of these fluoroquinolones against Gram-positives are weaker than against most Gram-negatives (Table 1). Thus, clinically resistant mutants of S. aureus can be obtained not only in a single step in vitro but also frequently from patients receiving fluoroquinolone therapy. Molecular genetic analysis of the underlying mechanism of resistance in S. aureus revealed that topo IV is the primary, more sensitive target, while DNA gyrase is secondary (62). One approach to improve the activity against Gram-positives was the chemical modification of the C8 position: Introducing a -F, a -Cl, or a -OCH3 substituent yielded...
Inhibitors of Bacterial Topoisomerases: Mechanisms of Action and Resistance and Clinical Aspects

Planta Med 67 (2001) 9

fluoroquinolones, like sparfloxacin, sitafloxacin, and moxifloxacin (Fig. 4) with enhanced activities against Gram-positive and several mycobacterial species, while retaining most of the activities against Gram-negatives (Table 1). However, a halogen substituent is associated with phototoxicity limiting the use of, e.g., sparfloxacin and ciprofloxacin (63). In contrast, no such side effects have been reported for moxifloxacin with a -OCH₃ substitution.

In vitro data indicate that topo IV is the primary target of fluoroquinolones in Enterococcus faecalis (64), (65) and S. pneumoniae, too (66), although for S. pneumoniae the target preference seems to depend on the structure of the fluoroquinolone (67).

Resistance due to Altered Drug Transport

Accumulation of fluoroquinolones in the cytoplasm at relevant concentrations is due to both the chelating activity of the 4-oxo-3-carboxylic acid moiety, which destabilizes the lipopolysaccharide layer of the outer membrane (68), and the zwitterionic character of these drugs, allowing them to pass the cytoplasmic membrane by exploiting the proton gradient (Fig. 4) (69).

Drug accumulation in a Gram-negative bacterial cell can be reduced by two different mechanisms: (i) reduction of the drug influx into the cell through the outer membrane, the major barrier for hydrophilic molecules, and (ii) increase in drug efflux out of the cell by energy-driven transmembrane export (Fig. 7) (70).

Some less hydrophilic quinolones like nalidixic acid are assumed to partially diffuse directly through the lipid bilayer of the outer membrane (71). However, diffusion of small (Mᵣ < 700) hydrophilic molecules, usually nutrients, occurs through porins, channel-forming proteins in the outer membrane. One of the best studied porins is the trimeric OmpF porin of E. coli, which is the major entry site for fluoroquinolones (72). Beside various environmental factors, e.g., osmolarity, temperature, pH, which can modulate the expression of OmpF (73), mutations abolishing the function and/or the expression of OmpF have been shown to result in reduced susceptibility to fluoroquinolones and unrelated drugs (74), (75). Compared to a gyrA mutation the loss of OmpF porin function has less impact on the quinolone resistance (76).

Increased drug export has been identified as a widely distributed mechanism of broad-spectrum resistance to antibiotics. One of the best studied systems of antibiotic export is that controlled by the mar operon of E. coli (77) which negatively controls the expression of OmpF and positively controls the expression of the efflux pump AcrAB-ToIC (Fig. 7). This pump is located to the RND efflux systems, one of four known major classes of transport systems. Several homologous efflux pumps involved in quinolone resistance have been identified in Gram-negative enterobacteria (78), Neisseria gonorrhoeae (79), and P. aeruginosa (80), (81), (82), (83). While efflux pumps significantly contribute to the natural multiple-drug resistance of and have a high impact on fluoroquinolone resistance in P. aeruginosa, their role for quinolone resistance in other Gram-negatives is less well established (84). Increased efflux is found in clinical isolates as well as in laboratory mutants, but is associated with a much lesser increase in resistance compared to target-mediated mutations (85). The genetic basis for increased efflux is poorly understood and in E. coli mutations affecting the global regulatory component MarR or local repressors, like AcrR, can be involved (Fig. 7) (86).

In Gram-positive drug efflux pumps belonging to the major facilitator family (87) have also been identified as factors modulating the fluoroquinolone resistance. These include NorA of S. aureus (88), (89) and PmrA of S. pneumoniae (90), which can be inhibited by reserpine and predominantly affect the activities of hydrophilic compounds, like norfloxacin and ciprofloxacin, but not those of more hydrophobic derivatives, like sparfloxacin or moxifloxacin (91).

Outlook

Within 15 years the fluoroquinolone class of antibacterial agents has become one of the most important drug classes showing high bactericidal activity against a broad range of Gram-positive and Gram-negative pathogens. Combined with favorable pharmacokinetics the pharmacodynamic features of the fluoroquinolones made these drugs most powerful chemotherapeutic agents for nearly all kinds of infections. However, clinically relevant resistance has developed even in strains belonging to highly susceptible species, most probably due to a high selective pressure by inappropriate use of these drugs. Thus, strictly regulated indications for use are necessary to avoid rapid development of resistance and to conserve this potential for the future. While the synthetic quinolone antimicrobial drugs are advantageous over naturally occurring by lack of both enzymatic inactivation mechanism and
transferable resistance it would be naive thinking that development of resistance is impossible. A promising way of saving the antimicrobial potential of new drugs is their prudent use for strictly defined indications and a continuing monitoring of resistance development.

References

Inhibitors of Bacterial Topoisomerases: Mechanisms of Action and Resistance and Clinical Aspects

Planta Med 67 (2001) 11


Prof. Dr. Peter Heisig
Pharmazeutische Biologie Universität Hamburg
Bundesstraße 45
20146 Hamburg
Germany
E-mail: heisig@chemie.uni-hamburg.de
Fax: +49 (0)40-42838-3895
Phone: +49 (0)40-42838-3899