

Summary:

Fluoroquinolones interfere with bacterial DNA metabolism by the inhibition of two enzymes, Topoisomerase II (syn. DNA gyrase) and Topoisomerase IV. In Gram-negative organisms DNA gyrase is the primary target, whereas in Gram-positive bacteria Topoisomerase IV was recently found to be most affected.

The function of DNA gyrase is to introduce supercoils into the linear DNA double helix, which results in the highly condensed 3-dimensional structure of the DNA usually present inside the cell. The function of Topoisomerase IV still is barely understood.

Models to explain the activity of quinolones at the target site only exist for DNA gyrase: During the supercoiling process, both DNA strands are cleaved by DNA gyrase at 4 base pair staggered sites, forming a "quinolone binding pocket".

Two quinolone molecules self-assemble inside the pocket in dimer structure and attach to the gyrase-DNA complex electrostatically, which stabilizes the intermediate stage of this reaction step.

Permanent gaps in the DNA strands induce synthesis of repair enzymes (exonucleases), initiating uncoordinated repair processes, which results in irreversible damage to the DNA and, finally, cell death.

Fluoroquinolones inhibit two enzymes of DNA metabolism in bacteria, Topoisomerase II and IV, the first one also being known as DNA gyrase.

Depending on the type of bacterium, these enzymes represent either the primary or secondary target of antimicrobial action. In Gram-negative bacteria, such as E.coli, fluoroquinolones predominantly inhibit DNA gyrase, whereas for Gram-positive organisms like Staph. aureus, Topoisomerase IV was recently found to be the principle target.

DNA gyrase and Topoisomerase IV have a very similar protein structure, each composed of two subunits (Gyr-A and Gyr-B). Their principal function is different:

The so-called DNA gyrase introduces negative supercoils into the linear DNA double helix, which results in the highly condensed 3-dimensional structure of the genetic material usually present inside the cell. This mechanism is necessary to condense the bacterial chromosome. In E. coli, for example, a DNA strand of around 1.300 µm length must fit into a cell which is only 2 µm long. The function of Topoisomerase IV is barely understood. However, it is known that this enzyme is involved in the separation process of the DNA daughter chains after chromosome duplication. Models to explain the activity of quinolones at the target site at present only exist for DNA gyrase.

The Gyr-A subunits of this enzyme were proposed to initially bind to the double stranded DNA helix. In an ATP-dependent process, described as "intermediate gate opening step", both DNA strands are cleaved at certain 4 base pair staggered sites. The 5' ends of the DNA chain are thereby bound covalently to Tyrosin₁₂₂ residues within the Gyr-A subunits. Gyr-B subunits are probably responsible for the ATP-dependent resealing process of the DNA.

Mechanism of Action**2/4**

At the location described above, DNA is present as single strands, forming a bubble-shaped binding pocket. Two quinolone molecules self-assemble to form a dimer structure inside the gyrase-induced DNA enzyme pocket. They bind to the complex by electrostatic forces, which stabilizes the intermediate stage in this reaction step. Evidence exists that the C7-amine substituents of quinolones additionally interact with proposed “quinolone binding pockets”, located at the Gyr-B subunits, in order to further strengthen the attachment to the drug-DNA-enzyme complex. In this way the progress of the supercoiling procedure, which would include rearrangement of the DNA segments, reattachment and resealing of the cuts, is locked up.

Permanent gaps in the DNA strands induce synthesis of repair enzymes called exonucleases, initiating uncoordinated repair processes. This results in breakdown of the DNA, leading to irreversible damage and, finally, to death of the bacterium.

References:

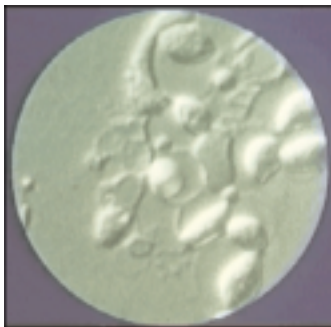
- (1) Hooper DC, Wolfson JS: Mechanism of quinolone action and bacterial killing, in Hooper DC, Wolfson JS (eds): Quinolone Antimicrobial Agents, ed 2. Washington DC, American Society for Microbiology: 53 - 75, 1993.
- (2) Shen LL: Quinolone - DNA interaction, in Hooper DC, Wolfson JS (eds): Quinolone Antimicrobial Agents, ed 2. Washington DC, American Society for Microbiology: 77 - 95, 1993.
- (3) Morais Cabral JH, Jackson AP, Smith CV, Shikotra N, Maxwell A, Liddington RC: Crystal structure of the breakage-reunion domain of DNA gyrase. Nature Vol 388 / 28: 903 - 906, 1997.

Mechanism of Action / Effect on Bacteria

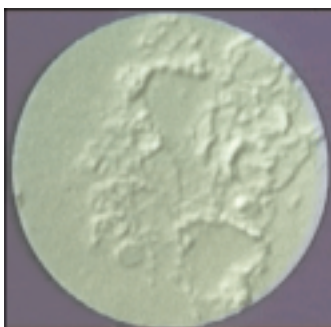
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Start frame shows intact, mainly rod-shaped bacteria (E. coli).



After application of Enrofloxacin bacteria have started to swell (circular shape). Some bacteria have already burst (spots).



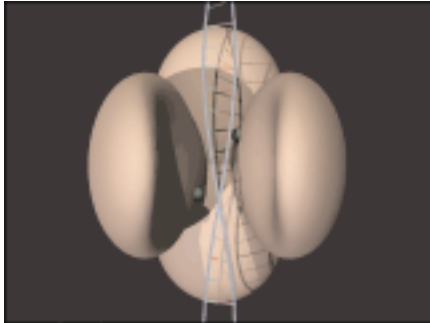
After 7 hours all bacteria have bursted (spots).

The video was taken at Bayer Laboratories, Wuppertal, Germany.
Microscopic resolution was 1000 x.

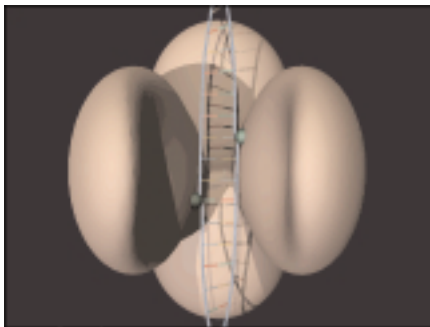
Bacteria are E. coli. Real time between first and last frame was approx. 7 h.

Mechanism of Action / DNA gyrase Inhibition

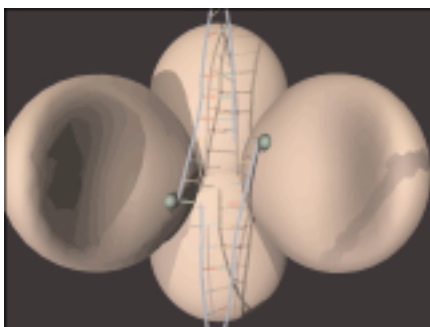
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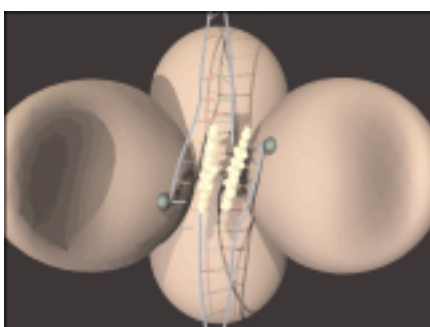
DNA double helix, base pairs coloured, and gyrase with two A and two B subunits.



DNA strings bind covalently to Tyrosine₁₂₂ molecules at DNA gyrase A subunits.



DNA strings are disconnected.



Enrofloxacin molecules (Baytril) bind to “quinolone binding pocket”.