Mechanism of action of fluoroquinolones: the basics...

DNA

PORIN

Gram (-)

DNA gyrase

Gram (+)

Topo isomerase
2 key enzymes in DNA replication:

DNA gyrase

- Stabilize positive node
- Break back segment
- Reseal break on front side

Topoisomerase IV

Bacterial DNA is supercoiled
Ternary complex
DNA - enzyme - fluoroquinolone

FLUOROQUINOLONES:
4 stacked molecules

DNA GYRASE catalytic subunits

COVALENTLY CLOSED CIRCULAR DNA

DNA GYRASE ATP binding subunits

(Shen, *in* Quinolone Antimicrobial Agents, 1993)
Ternary complex
DNA - enzyme - fluoroquinolone

COVALENTLY CLOSED CIRCULAR DNA

DNA GYRASE catalytic subunits

DNA GYRASE ATP binding subunits

FLUOROQUINOLONES: 4 stacked molecules

Cabral et al., Nature, 1997
Resistance to fluoroquinolones: the basics

- Decreased permeability
- Efflux pump
- Mutation of enzymes

Gram (-) vs. Gram (+)
Fluoroquinolones are the first entirely man-made antibiotics: do we understand our molecule?

Don’t panic, we will travel together....
Chemistry and Activity

This is where all begins...
The pharmacophore common to all fluoroquinolones

[Diagram of fluoroquinolone structure with labels for binding to DNA, the enzyme, and auto-assembling domain (for stacking).]
From chloroquine to nalidixic acid...

Chloroquine (1939)

7-chloroquinoline (1958, synthesis intermediate found to display antibacterial activity)

Nalidixic acid (1962)
Nalidixic acid *

- typical chemical features of fluoroquinolones (a, b, c)
  BUT a naphthridone
  (N at position 8: ▲)

- limited usefulness as drug
  - narrow antibacterial spectrum
    (Enterobacteriaceae only)
  - short half-life (1.5h)
  - high protein binding (90%)

* Belg. pat. 612,258 to Sterling Drugs, 1962
From nalidixic acid to the 1st fluoroquinolone (1 of 4)

nalidixic acid

oxolinic acid *

1. modify naphthyridone into quinolone

shows reduced protein binding...

* Ger. pat. to Warner Lambert, 1967

* quinoleine
From nalidixic acid to the 1st fluoroquinolone (1 of 4)

2. discovery of flumequine *

* Ger pat. to Rikker Labs, 1973

flumequine *

shows weak but broad Gram(-) activity

* benzo-quinolizine
From nalidixic acid to the 1st fluoroquinolone (1 of 4)

3. introduce a piperazine *

shows longer half-life...

* Ger. Pat. to Roger Bellon, 1974

* pyrido-2-3-pyrimidine
From nalidixic acid to the 1st fluoroquinolone (1 of 4)

combine all 3 features *

1978

broader Gram(-) activity
less protein binding (50%)
longer half-life (3-4h)

* Belgian patent 863,429, 1978 to Kyorin

* 6-fluoro-7-pyrimidino-quinoleine
From norfloxacin to the other 1st generation fluoroquinolones: pefloxacin

Add a methyl to still increase half-life

*n* Ger. pat. 2,840,910 to Roger Bellon/Dainippon, 1979
From norfloxacin to the other 1st generation fluoroquinolones: ofloxacin

* Eur. pat. Appl. 47,005 to Daiichi, 1982
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From norfloxacin to the other 1st generation fluoroquinolones: ciprofloxacin

norfloxacin

ciprofloxacin *

cyclopropyl to increase potency

pefloxacin

ofloxacin

* Ger. pat. 3,142,854 to Bayer AG, 1983
"1st generation" fluoroquinolones

- Norfloxacin

- Pefloxacin

- Ciprofloxacin

- Ofloxacin
The "first generation" of fluoroquinolones

- Norfloxacin
- Pefloxacin
- Ofloxacin
- Ciprofloxacin
- Fleroxacin
- Rufloxacin

Improved anti Gram (-) activity

$t_{1/2}$ activity

- Norfloxacin: 3-4 h ++
- Pefloxacin: 11 h +
- Ofloxacin: 6 h ++
- Ciprofloxacin: 3-4 h +++

- Nalidixic acid
- Oxolinic acid
- Cinoxacin
- Pipemidic acid
From ofloxacin to levofloxacin...

Ofloxacin is a racemic mixture

The active form of ofloxacin is the (-) S isomer

* Eur. pat. 206,283 to Daiichi, 1987
The present "first generation" of fluoroquinolones ...


- Nalidixic acid
- Oxolinic acid
- Flumequine
- Pipemidic acid

• Norfloxacin
• Pefloxacin
• Ofloxacin
• Ciprofloxacin
  - Fleroxacin
  - Rufloxacin

improved anti Gram (-) activity

<table>
<thead>
<tr>
<th>Activity</th>
<th>t_{1/2}</th>
</tr>
</thead>
<tbody>
<tr>
<td>++</td>
<td>3-4 h</td>
</tr>
<tr>
<td>+</td>
<td>11 h</td>
</tr>
<tr>
<td>++</td>
<td>6 h</td>
</tr>
<tr>
<td>+++</td>
<td>3-4 h</td>
</tr>
<tr>
<td>+++++</td>
<td>6 h</td>
</tr>
</tbody>
</table>

Levofloxacin

6 h +++++

twice as active as ofloxacin per g
How to improve the chemotherapeutic usefulness of the "first generation" fluoroquinolones

1. Maintain broad Gram(-) activity
2. Improve Gram(+) activity
3. Acquire activity against anaerobes

“2d generation”

“3d generation”
The “second generation” fluoroquinolones

- Temafloxacin $^a$
- Sparfloxacin $^b$
- Grepafloxacin $^c$
- Gatifloxacin $^d$

- Gram (-);
- improved Gram (+)

anti-anaerobe

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The “third generation” fluoroquinolones

- Trovafloxacin \(^b\)
- Moxifloxacin \(^c\)
- Gemifloxacin \(^d\)

Activity against *S. pneumoniae*

- **Ciprofloxacin** 0.5 - 2
- **Sparfloxacin** 0.125 - 0.5
- **Temafloxacin** 0.5 - 1
- **Moxifloxacin** 0.01 - 0.5
- **Trovafloxacin** 0.007 - 0.25
Resistance au fluoroquinolones : les mécanismes de base ...

perméabilité diminuée

pompes à efflux

mutation des enzymes cibles

Gram (-)

Gram (+)

perméabilité diminuée

pompes à efflux

mutation des enzymes cibles

Gram (-)

Gram (+)
Resistance au fluoroquinolones : rôle des mutations au niveau de la cible

Gram (-)

Gram (+)

Élévation des CMI de 3 à 5 dilutions (≈ 20 X) par mutation

mutation des enzymes cibles
Is there a SAR for emergence of resistance?

The "Mutant Prevention Concentration" *

"When Mycobacterium bovis BCG and Staphylococcus aureus were plated on agar containing increasing concentrations of fluoroquinolone, colony numbers exhibited a sharp drop, followed by a plateau and a second sharp drop.

The plateau region correlated, with the presence of first-step resistant mutants. Mutants were not recovered at concentrations above those required for the second sharp drop, thereby defining a mutant prevention concentration (MPC).

A C8-methoxy group lowered the MPC for an N-1-cyclopropyl fluoroquinolone"
Is there a SAR for emergence of resistance?

Bactericidal activity of FQs against *Mycobacterium bovis*.

- **SAR**
  - **R** = HR = OCH₃
  - **N**
  - **C**
  - **O**
  - **F**
  - **N**
  - **O⁻**
  - **OCH₃**
  - **HN**
  - **H₅C₂**

<table>
<thead>
<tr>
<th>FQ Concentration</th>
<th>Fraction of Survivors</th>
</tr>
</thead>
<tbody>
<tr>
<td>10⁻¹⁰</td>
<td>10⁻⁸</td>
</tr>
<tr>
<td>10⁻⁸</td>
<td>10⁻⁶</td>
</tr>
<tr>
<td>10⁻⁶</td>
<td>10⁻⁴</td>
</tr>
<tr>
<td>10⁻⁴</td>
<td>10⁻²</td>
</tr>
<tr>
<td>10⁻²</td>
<td>10</td>
</tr>
</tbody>
</table>

- **MIC** (99)
  - 0.25
- **MPC** (10)
  - 0.9
- **MPC/MIC**
  - 3.6

- **PD160793**
- **PD161148**

- **R** = OCH₃
- **R** = H

- Dong *et al*; AAC 43:1756-1758

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Fluoroquinolones with a C8-methoxy

I

II

III

ciprofloxacin
gatifloxacin
moxifloxacin

Not in Belgium
Yes
Toxicity

This is where all may fail...
Frequent side effects of fluoroquinolones: is there a SAR?

- COMPLEXATION WITH METALLIC IONS (Fe, Al, Mg, Ca)
- PHOTOTOXICITY
- DRUG INTERACTIONS: INHIBITION OF cyt P450 (1A2)
- CNS TOXICITY (BINDING TO GABA RECEPTOR)
- GASTRO-INTESTINAL DISCOMFORT
- CARTILAGE and MUSCULOSQUELETAL TOXICITY
SAR of frequent side effects

- Binding to GABA receptor
- Penetration in CNS
- Inhibition of P450
- Phototoxicity
- Ca++, Al+++, Fe++ complexation
- All FQs
- sparflo, flero, lomeflo
- Inhibition of P450
Fluoroquinolone with low or no drug interactions.

Moxifloxacin

Yes
Rare side effects of fluoroquinolones:

- **RENAL TOXICITY**
crystalluria, hematuria, interstitial nephritis, acute renal failure

- **CARDIAC TOXICITY** (QT prolongation, *Torsades de pointe*)

- **HEPATOTOXICITY**
temafloxacin syndrome / trovafloxacin syndrome
Pharmacokinetics

This is where people start sleeping..
SAR of pharmacokinetic parameters

Bulky substituants increase $t_{1/2}$.

Cipro, gati, moxi.

Peflo, oflo, gati, moxi.

Increased $V_d$.

Fluoroquinolones

$\text{SAR of pharmacokinetic parameters}$

$\text{Bulky substituants}$

$\text{cipro, gati, moxi}$

$\text{peflo, oflo, gati, moxi}$

$\text{Increased V_d}$
### SAR of main pharmacokinetic parameters: how to get a long half life

<table>
<thead>
<tr>
<th>Drug</th>
<th>$t_{1/2}$ (h)</th>
<th>no. of daily administrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>oflo / lévo</td>
<td>5 - 7</td>
<td>2 x*</td>
</tr>
<tr>
<td>peflo</td>
<td>10</td>
<td>2 x*</td>
</tr>
<tr>
<td>flero</td>
<td>9 - 13</td>
<td>1 x</td>
</tr>
<tr>
<td>grepa</td>
<td>10 - 12</td>
<td>1 x</td>
</tr>
<tr>
<td>gati</td>
<td>13</td>
<td>1 x</td>
</tr>
<tr>
<td>gemi</td>
<td>8</td>
<td>1 x</td>
</tr>
<tr>
<td>trova</td>
<td>10</td>
<td>1 x</td>
</tr>
<tr>
<td>moxi</td>
<td>12</td>
<td>1 x</td>
</tr>
<tr>
<td>other FQ</td>
<td>3 - 6</td>
<td>2 x</td>
</tr>
</tbody>
</table>

* higher MIC...
“Inadequate dosing of antibiotics is probably an important reason for misuse and subsequent risk of resistance. A recommendation on proper dosing regimens for different infections would be an important part of a comprehensive strategy. The possibility to produce such a dose recommendation based on pharmacokinetic and pharmacodynamic considerations will be further investigated in one of the CPMP working parties…”

European Agency of the Evaluation of Medicinal Products (London)

EMEA discussion paper on Antimicrobial resistance
3 January 1999 EMEA/9880/99
### Pharmacokinetic parameters in relation with efficacy

<table>
<thead>
<tr>
<th>Dose (mg)</th>
<th>Cmax (mg/l)</th>
<th>MIC for pk/MIC=10</th>
<th>AUC (mg.h/l)</th>
<th>MIC for AUIC=125</th>
</tr>
</thead>
<tbody>
<tr>
<td>norfloxacin</td>
<td>400 (X2)</td>
<td>1.6</td>
<td>0.2</td>
<td>14</td>
</tr>
<tr>
<td>pefloxacin</td>
<td>400 (X2)</td>
<td>4.6</td>
<td>0.4</td>
<td>108</td>
</tr>
<tr>
<td>ciprofloxacin</td>
<td>500 (X2)</td>
<td>1.5</td>
<td>0.2</td>
<td>17</td>
</tr>
<tr>
<td>ofloxacin</td>
<td>200 (X2)</td>
<td>3.1</td>
<td>0.4</td>
<td>66</td>
</tr>
<tr>
<td>levofloxacin</td>
<td>500</td>
<td>5.0</td>
<td>0.5</td>
<td>47</td>
</tr>
<tr>
<td>moxifloxacin</td>
<td>400</td>
<td>4.5</td>
<td>0.4</td>
<td>48</td>
</tr>
</tbody>
</table>
Optimizing dosage for fluoroquinolones

increase the amount administered, in order to optimize AUC/MIC should be > 125
and peak/MIC should be > 10

Get both a peak and a AUC!!
How to apply this?

Levofloxacin
- 500 mg
- 1X/day
- AUC [(mg/l) x h] 47
- Peak [mg/l] 5
- MICmax < 0.5

Moxifloxacin
- 400 mg
- 1X/day
- AUC [(mg/l) x h] 48
- Peak [mg/l] 4.5
- MICmax < 0.5

MIC data: J. Verhaegen et al., 2001

% of sensitive strains

 MIC

0 0.015 0.03 0.06 0.125 0.25 0.5 1 2 4

0 20 40 60 80 100

moxi

levo

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Take home” message

- Dosage is key to success
- Dosage should match bacterial sensitivity
- peak, AUC/MIC are keys to success
- use a single, appropriate dose for long-life fluoroquinolones (moxifloxacin), or
- repeat the dose for short-lived fluoroquinolones (all others so far…)
- for fluoroquinolones, the limit is an MIC of 0.5 µg/ml