FLUOROQUINOLONES: from structure to activity and toxicity

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www.sbimc.org - www.bvikm.org

soon...
Mechanism of action of fluoroquinolones: the basics...

DNA

PORIN

Gram (-)

DNA gyrase

Topo isomerase

Gram (+)
2 key enzymes in DNA replication:

- DNA gyrase
- Topoisomerase IV

bacterial DNA is supercoiled
Ternary complex
DNA - enzyme - fluoroquinolone

COVALENTLY CLOSED CIRCULAR DNA

DNA GYRASE catalytic subunits

DNA GYRASE ATP binding subunits

FLUOROQUINOLONES:
4 stacked molecules

(Shen, *in* Quinolone Antimicrobial Agents, 1993)
Resistance to fluoroquinolones: the basics

- Decreased permeability
- Efflux pump
- Mutation of the enzymes

DNA gyrase
Topoisomerase

Gram (-)
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
From chloroquine to nalidixic acid...

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

1. modify naphthyridone into quinolone

* quinoleine

* Ger. pat. to Warner Lambert, 1967
From nalidixic acid to the 1st fluoroquinolone (1 of 4)

2. discovery of flumequine *

shows weak but broad Gram(-) activity

* Ger pat. to Rikker Labs, 1973

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

nalidixic acid

\[
\begin{array}{c}
\text{N} \\
\text{O} \\
\text{C} \\
\text{C}_2\text{H}_5 \\
\text{O}^- \\
\text{N} \\
\text{O} \\
\text{C}_2\text{H}_5 \\
\text{O}^- \\
\text{N} \\
\text{O} \\
\text{C}_2\text{H}_5 \\
\text{O}^- \\
\end{array}
\]

3. introduce a piperazine *

pipemidic acid *

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

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

norfloxacin

tricyclic compound
(as in flumequine but morpholine ring)

pefloxacin

ofloxacin*

* Eur. pat. Appl. 47,005 to Daiichi, 1982
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
- ciprofloxacin
- pefloxacin
- ofloxacin
The "first generation" of fluoroquinolones

- Nalidixic acid
- Oxolinic acid
- Cinoxacin
- Pipemidic acid

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

Improved anti Gram (-) activity

<table>
<thead>
<tr>
<th>$t_{1/2}$</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-4 h</td>
<td>++</td>
</tr>
<tr>
<td>11 h</td>
<td>+</td>
</tr>
<tr>
<td>6 h</td>
<td>++</td>
</tr>
<tr>
<td>3-4 h</td>
<td>+++</td>
</tr>
</tbody>
</table>
Ofloxacin is a racemic mixture

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

Levofloxacin is the pure (-) 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

**t$_{1/2}$** activity

<table>
<thead>
<tr>
<th>Compound</th>
<th>t$_{1/2}$</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norfloxacin</td>
<td>3-4 h</td>
<td>++</td>
</tr>
<tr>
<td>Pefloxacin</td>
<td>11 h</td>
<td>+</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>6 h</td>
<td>++</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>3-4 h</td>
<td>+++</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>6 h</td>
<td>++++</td>
</tr>
</tbody>
</table>

Levofloxacin is 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

---

\(^a\): Toyama, 1988 (?) ; \(^b\): Dainippon, 1985-1987; \(^c\): Otskuda, 1989; \(^d\): Kyorin, 1988
The “third generation” fluoroquinolones

- Clinafloxacin\textsuperscript{a}
- Trovafloxacin\textsuperscript{b}
- Moxifloxacin\textsuperscript{c}
- Gemifloxacin\textsuperscript{d}

anti-Gram (-)
anti-Gram (+)
anti-anaerobe

\textsuperscript{a}: Kyorin, 1987; \textsuperscript{b}: Pfizer, 1993; \textsuperscript{c}: Bayer, 1994; \textsuperscript{d}: LG Chemical Ltd., S. Korea, 1994-98
1. maintenance of anti-Gram (-) activity

- **Gram (-)**
- **NH₂**
- **piperazine**
- **Cl, F**
- **O-CH₃**
- **bulky group**
- **halogen methoxy**
- **MIC**
Gram (-) activity (E. coli)

I

II

ciprofloxacin
0.125 - 0.5

grepafloxacin
0.06 - 2

gatifloxacin
0.06
2. improving Gram (+) activity (S. pneumoniae)

- **Gram (+)**
- **CH₃**
- **pyrimidine**
- **H₂N**
- **N**
- **naphthyridone**
- **if X₈ = C**
- **Cl, F** halogen
- **O-CH₃** methoxy
- **bulky group**
Activity against *S. pneumoniae*

I: ciprofloxacin
   0.5 - 2

II: sparfloxacin
   0.125 - 0.5
   temafloxacin
   0.5 - 1

III: moxifloxacin
    0.01 - 0.5
    trovafloxacin
    0.007 - 0.25
3. obtaining activity against anaerobes ...

R_{1}\quad R_{5} \quad COOH

\begin{align*}
X_{8} & = \text{C} \\
N & \quad \text{naphthyridone} \\
O-\text{CH}_{3} & \quad \text{methoxy} \\
\text{bulky group} &
\end{align*}
Activity against *B. fragilis*

<table>
<thead>
<tr>
<th>Compound</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td>2 - 128</td>
</tr>
<tr>
<td>Gatifloxacin</td>
<td>0.25 - 8</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>0.25 - 8</td>
</tr>
<tr>
<td>Gemifloxacin</td>
<td>0.5 - 64</td>
</tr>
<tr>
<td>Trovafloxacin</td>
<td>0.125 - 8</td>
</tr>
</tbody>
</table>
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*

<table>
<thead>
<tr>
<th>FQ concentration (FQ concentration)</th>
<th>Fraction of survivors</th>
</tr>
</thead>
<tbody>
<tr>
<td>10^-10</td>
<td>1.0</td>
</tr>
<tr>
<td>10^-8</td>
<td>1.0</td>
</tr>
<tr>
<td>10^-6</td>
<td>1.0</td>
</tr>
<tr>
<td>10^-4</td>
<td>1.0</td>
</tr>
<tr>
<td>10^-2</td>
<td>1.0</td>
</tr>
<tr>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

**MIC (99)**

- PD160793: 0.25
- PD161148: 0.8

**MPC (10)**

- PD160793: 0.9
- PD161148: 9

**MPC/MIC**

- PD160793: 3.6
- PD161148: 12

Dong *et al*; AAC 43:1756-1758
Fluoroquinolones with a C8-methoxy

I

II

ciprofloxacin
gatifloxacin

III

moxifloxacin
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
- Cipro, grepa...
Fluoroquinolones with low or no drug interactions..
Fluoroquinolones with high phototoxicity ...

Fleroxacin\textsuperscript{a}  

Lomefloxacin\textsuperscript{b}  

Bay 3118\textsuperscript{c}

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
Rare side effects of fluoroquinolones: cardiac toxicity

*Torsade de pointes*: paroxysm of ventricular tachycardia in which the electrocardiogram shows a steady undulation in the QRS axis in runs of 5 to 20 beats with progressive changes in direction. It is a most severe type of arrhythmia which may cause death. It is most often associated with and preceded by a prolongation of the QT interval.

<table>
<thead>
<tr>
<th>Change in QTc prolongation (msec)</th>
<th>Drug Combinations</th>
<th>Population Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>fluoxetine: 2</td>
<td>moxifloxacin: 7</td>
</tr>
<tr>
<td>20 msec</td>
<td>grepafloxacin: 10</td>
<td>sparflloxacin: 15</td>
</tr>
<tr>
<td>15</td>
<td>cisapride + clari: 25</td>
<td>terfenadine: 46</td>
</tr>
<tr>
<td>50</td>
<td></td>
<td>20 msec: population risk</td>
</tr>
</tbody>
</table>
Cardiac toxicity QT prolongation: is there any SAR?

cisapride

astemizole

terfenadine
Cardiac toxicity QT prolongation: is there any SAR?

- **cisapride**
  - ![cisapride molecule](image)

- **sparfloxacin**
  - ![sparfloxacin molecule](image)

- **grepafloxacin**
  - ![grepafloxacin molecule](image)
Other severe toxicities

1992:

The temafloxacin syndrome:

*hemolytic uraemic anemia*

- discoloured urine, fever
- jaundice, nausea, vomiting
- abdominal pain
- coagulopathy
- hepatic and renal dysfunction

- 0.056% incidence
- 2 deaths

withdrawn in June 1992

1999:

The trovafloxacin syndrome:

*serious hepatic events*

- laboratory abnormallities
  - ALT, bilirubin, encephalopathies
  - necrotic inflammation

- 0.0056% incidence
- 5 transplants
- 6 deaths (multifactorial)

withdrawn / limited in June 1999
Which part of the molecule is the culprit?

withdrawn in June 1992

withdrawn / limited in June 1999
Pharmacokinetics

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

Bulky substituent

$t_{1/2}$

Biodisponibility

cipro, grepa, gati, gemi, moxi

flero, peflo, oflo, grepa, gati, trova, moxi, gemi

gemi, trova
### 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</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...
SAR of main pharmacokinetic parameters: biodisposonibility

<table>
<thead>
<tr>
<th></th>
<th>biodisponibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>trovafloxacin</td>
<td>90 %</td>
</tr>
<tr>
<td>no data available for gemifloxacin</td>
<td></td>
</tr>
<tr>
<td>other FQ</td>
<td>60-90 %</td>
</tr>
</tbody>
</table>
**SAR of main pharmacokinetic parameters: volume of distribution**

<table>
<thead>
<tr>
<th></th>
<th>$V_d$ (L/Kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ciprofloxacin</td>
<td>3</td>
</tr>
<tr>
<td>gatifloxacin</td>
<td>1.8</td>
</tr>
<tr>
<td>grepafloxacin</td>
<td>8</td>
</tr>
<tr>
<td>gemifloxacin</td>
<td>1.5</td>
</tr>
<tr>
<td>moxifloxacin</td>
<td>2</td>
</tr>
<tr>
<td>other FQ</td>
<td>1-1.5</td>
</tr>
</tbody>
</table>
Resistance: do not forget the correct dosing...

“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) AUIC=125</th>
<th>MIC for AUIC=125</th>
</tr>
</thead>
<tbody>
<tr>
<td>norflo</td>
<td>400 (X2)</td>
<td>1.6</td>
<td>0.2</td>
<td>14</td>
</tr>
<tr>
<td>peflo</td>
<td>400 (X2)</td>
<td>4.6</td>
<td>0.4</td>
<td>108</td>
</tr>
<tr>
<td>cipro</td>
<td>500 (X2)</td>
<td>1.5</td>
<td>0.2</td>
<td>17</td>
</tr>
<tr>
<td>oflo</td>
<td>200 (X2)</td>
<td>3.1</td>
<td>0.4</td>
<td>66</td>
</tr>
<tr>
<td>levoflo</td>
<td>500</td>
<td>8.7</td>
<td>0.8</td>
<td>73</td>
</tr>
<tr>
<td>grepa</td>
<td>600</td>
<td>1.4</td>
<td>0.1</td>
<td>20</td>
</tr>
<tr>
<td>gati</td>
<td>400</td>
<td>4.5</td>
<td>0.4</td>
<td>28</td>
</tr>
<tr>
<td>trova</td>
<td>200</td>
<td>2.3</td>
<td>0.2</td>
<td>25</td>
</tr>
<tr>
<td>moxi</td>
<td>400</td>
<td>2.5</td>
<td>0.2</td>
<td>30</td>
</tr>
<tr>
<td>gemi</td>
<td>600</td>
<td>4</td>
<td>0.4</td>
<td>24</td>
</tr>
</tbody>
</table>
Consider local epidemiology, and do not believe it is always a first choice...

The use of the FQ should focus on infections in which
- there is a differential benefit over conventional agents in terms of efficacy, safety, or cost;
- in infections for which few alternative treatments exist, or
- against organisms towards which they are sufficiently active to prevent the rapid emergence of resistance.
Shall we have a very bright future?

or some problems?