**New Fluoroquinolone Finafloxicin HCl (FIN): Route of Synthesis, Physicochemical Characteristics and Activity Under Neutral and Acidic Conditions**

S-E. Wohlert1, T. Jaetsch1, B. Galenkamp2, H. J. Knops2, N. Lu1, M. Preiss2, D. Haebic1, H. Labischinski2

1 MerLion Pharmaceuticals GmbH, Berlin, Germany. 2 Bayer AG, Leverkusen, Germany.

**Methods**

Chemistry: Finafloxicin HCl (FIN, III) was synthesized by combining MOPY- (15.65) Morpholine-pyrrolidine (i), with Cyano-FQA, 7-chloro-8-cyano-1-cyclopropyl-6-fluoro-4-ethyl-3-quinolinecarboxylic acid (ii), followed by crystallization to the hydrochloride in two steps with ~55% overall yield. The two components, MOPY (i) and Cyano-FQA (ii) were prepared in 7-step syntheses each, with ~25% yield and 30% yield, respectively. The synthesis of FIN (III) started from 2-lithiodiol (i) and 1-pyrazolium-2-thione to form 1-tosylajimine (ii), which was converted into epoxide (iii) by 3-chloro-perbenzoic acid. Crystallization was introduced by opening the epoxide ring with SOCl2-phenylhydrazine and retrieval of the desired diastereomer (iv) by crystallization. The morpholine (v) was synthesized by acylation of (iv) with chloro-acetylchloride and subsequent cyclization. (v) was reduced to (vi) with a sodium borohydride boronfluoride-THF complex to give diazepine to (vii) and final hydrogenation to FIN (III). The Cyano-FQA (ii) synthesis started with fluoro-mylene (viii) reacting with (iv), which was eliminated to form hepta-chloro-xylene (ix) under UV-iradiation. Starting from (i0) formic acid (ix) and the corresponding cyanocarboxylic acid (ix) were subsequently formed before cyano-benzo-chloride (x) resulted from reacting with thionylchloide. Esterification of (xi) with ethyl-3-dimethylaminopropionate (β-DAAP), followed by reaction with cyclopropylamine followed by cyclization leads to (xii), which after acidic ester-dehydrolysis yield Cyano-FQA (ii).

**Results and Discussion**

Physicochemical characteristics of FIN (III).

**Description**

White to redish yellow substance

**Average molecular weight**

434.8546 g/mol 434.8546 (C21H18FN6O2)x HCl

**Optical purity**

αD20 = 120° (based on crude substance)

**Water content (25°C, 60% r.h., 40°C, 75% r.h., in PE bags)**

< 7.7% or ≥2x H2O per molecule FIN (III)

**Table 1. Solubility (at 25°C)**

<table>
<thead>
<tr>
<th>Solvent</th>
<th>FIN (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>4.6</td>
</tr>
<tr>
<td>pH 5.0</td>
<td>2.4</td>
</tr>
<tr>
<td>pH 7.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

**Table 2. Partition coefficients (P)**

<table>
<thead>
<tr>
<th>Solvent</th>
<th>FIN (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>64.0</td>
</tr>
<tr>
<td>pH 5.0</td>
<td>95.6</td>
</tr>
<tr>
<td>pH 7.0</td>
<td>97.0</td>
</tr>
</tbody>
</table>

**Table 3. Iodination constants (by potentiometric titration)**

<table>
<thead>
<tr>
<th>pH</th>
<th>β1</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 5.0</td>
<td>0.4</td>
</tr>
<tr>
<td>pH 7.0</td>
<td>0.6</td>
</tr>
</tbody>
</table>

**Polymerisation**

FIN (III) crystallized in at least 9 modifications (2 non solvated and 7 solvated forms) with the dithyline and an arachyline form as the most stable and preferred variants under ambient conditions.

**Antibacterial properties of FIN (III)**

**Figures 3 and 4, pH-dependent MIC**

**MIC (mg/L)**

<table>
<thead>
<tr>
<th>Organism</th>
<th>FIN (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>0.125</td>
</tr>
<tr>
<td>P. mirabilis</td>
<td>0.125</td>
</tr>
<tr>
<td>E. coli</td>
<td>0.03</td>
</tr>
<tr>
<td>P. mirabilis</td>
<td>0.03</td>
</tr>
<tr>
<td>E. coli</td>
<td>0.063</td>
</tr>
<tr>
<td>P. mirabilis</td>
<td>0.063</td>
</tr>
<tr>
<td>E. coli</td>
<td>0.016</td>
</tr>
<tr>
<td>P. mirabilis</td>
<td>0.016</td>
</tr>
<tr>
<td>E. coli</td>
<td>0.0078</td>
</tr>
<tr>
<td>P. mirabilis</td>
<td>0.0078</td>
</tr>
<tr>
<td>E. coli</td>
<td>0.06</td>
</tr>
<tr>
<td>P. mirabilis</td>
<td>0.06</td>
</tr>
</tbody>
</table>

**Conclusions**

• A scalable synthesis of the two building blocks and the novel fluoroquinolone finafloxicin hydrochloride (FIN, III) was established.

• The chemical structure of FIN (III) and important physicochemical characteristics were determined.

• The basic antibacterial activity of FIN (III) against Gram-negative and Gram-positive pathogens was determined.

• FIN (III) exhibited excellent and overall superior antibacterial activity at low pH, demonstrating an exceptional potential to treat infections in acidic environments, such as in the gastrointestinal or urogenital tract, in abscesses, intra-abdominal infections, TB, CF and others.

• In acidic environment FIN (III) inhibited relevant reference quinolones, most likely due to its lower intrinsic basic capacity enabling a more efficient uptake into the cells at lower pH.

**Literature**

Effect of pH on the In Vitro Activity of Finafloxacin against Gram-negative and Gram-positive Bacteria

M. KRESKEN1, B. KÖRBER-IRRGANG1, H. LABISCHINSKI2, W. STUBBINGS3

1Antifectives Intelligence GmbH, Rheinbach, Germany, 2Merlion Pharmaceuticals GmbH, Berlin, Germany, 3Merlion Pharmaceuticals Pte Ltd, Singapore

Revised Abstract

Introduction

Finafloxacin (FIN) is a novel 8-cyano-fluoroquinolone (FQ) that belongs to a new 8-cyano subclass. FIN contains a novel base component which confers improved antibacterial activity at slightly acidic pH (pH 5.0 – 6.0) where other FQs exhibit significantly reduced activity [1].

In addition, FIN exhibited superior activity to comparator FQs against adherent bacteria, in vitro, that was especially notable at low pH (2). FIN also exhibited superior activity in rodent infection models (3,4) which involved inflammation, abscess formation or other acidic foci of infection. The present study was performed to study the effect of the pH on the in vitro activity of FIN and CIP against 100 clinical isolates of various aerobic Gram-positive and Gram-negative bacterial species known to cause genito-urinary tract infections.

Results

Results are presented in Table 1. Overall, FIN exhibited the highest in vitro activity at acidic conditions, while CIP was most active at pH 7.3 or 8.0. MICs of FIN and CIP against CIP-S. e.coli are also illustrated in Figure 2 to demonstrate the opposing effect of pH on the inhibitory activity of these FQs.

Enterobacteriaeae: Median MICs of FIN for CIP-S isolates of E. coli, K. pneumoniae, P. mirabilis and M. morganii were ≤0.25 mg/L each at pH 0.0 and pH 6.0, 0.125-1 mg/L at pH 7.3 and 0.25-2 mg/L at pH 8.0. FIN was more active than CIP against CIP-S isolates of species at pH 5.0, while it was less active than CIP at pH 7.3 and 8.0. At pH 6.0, FIN showed superior activity to CIP against E. coli and K. pneumoniae and comparable activity to CIP against P. mirabilis, but was 4-fold less active than CIP against M. morganii. Similar differences were found for CIP-N.IS isolates.

P. aeruginosa: Based on median MICs, FIN showed comparable activity to CIP at acidic pH, but was less active than CIP at pH 7.3 and 8.0, respectively.

S. aureus: At pH 5.0 and 6.0, median MICs of FIN for CIP-S isolates (0.125 mg/L and 0.031 mg/L) were four dilution steps lower than those of CIP. Moreover, FIN was 2-fold more active than CIP at pH 5.0 and 8.0. This trend was also observed for CIP-N.IS isolates.

S. agilliciens: FIN demonstrated superior activity to CIP at pH 5.0 and 6.0 and showed equal activity at pH 7.3, while it was one dilution step less active at pH 8.0.

Conclusions

• FIN demonstrated superior activity to CIP under acidic conditions against isolates of all species, except P. aeruginosa for which both drugs showed similar potency under these conditions.

• FIN appears to be a promising new antimicrobial agent for the treatment of infections in acidic environments.

Literature

Antimicrobial Activity of Finafloxacin (FIN) against *Helicobacter pylori* In Vitro and In Vivo  


INSERM U853, Bordeaux, France, Bayer HealthCare AG, Elberfeld, Germany, MerLion Pharmaceuticals Pte Ltd, Singapore.

Introduction: FIN is a novel fluoroquinolone (FQ) belonging to a new 8-cyano subclass. FIN exhibits optimal efficacy at slightly acidic pH (5.0 - 6.0), under which other FQs lose activity. FIN is intended for therapeutic use against bacterial infections associated with an acidic environment such as *H. pylori* infection. The antibacterial activity of FIN was determined against FIN susceptible and resistant strains at acidic pH, and against *H. felis* in vivo.

Methods: *H. pylori* strains were obtained from patients gastroscoped in France. MICs for FIN and levofloxacin (LVX) were performed by agar dilution at 3 different pHs: 7.3, 6.3 and 5.3. The propensity for emergence of resistance in vivo was determined in a murine model in which *H. felis* was passaged until persistent infection was established that required triple therapy to eradicate.

Results: MICs and MC50 values of FIN and LVX for 31 isolates are shown in Table 1. Additionally, MICs were determined for a panel of 24 FQ-susceptible isolates (Table 2). Resistance rates of *H. pylori* isolates to FIN and LVX under standard conditions were investigated. In total, 25 *H. pylori* strains were investigated for their susceptibility to FIN and LVX. Initially, a panel of 21 strains was investigated. These were pre-defined as FIN susceptible (n = 18) or resistant (n = 3), based on their susceptibility to LVX.

Conclusions: FIN exhibited increased efficacy at acidic pH compared to other FQs against various *H. pylori* strains. This study confirms the in vitro activity of FIN in a wide range of species under acidic conditions. The in vivo efficacy of FIN against *H. pylori* was demonstrated in a murine model of persistent *H. pylori* infection. FIN may be a promising treatment that could improve *H. pylori* eradication therapy in humans.

**References:**
OBJECTIVES
MIC testing of aerobic bacteria with finafloxacin (FIN) at acidic and neutral pH was studied by comparing Etest®, CLSI agar dilution (AD) and broth microdilution (BMD) methods.

INTRODUCTION
Finafloxacin is a novel broad spectrum fluoroquinolone that exhibits optimal activity at slightly acidic conditions (pH 5.6) where other fluoroquinolones lose some of their activity. Hence, finafloxacin is intended for therapy of bacterial infections associated with an acidic environment such as *H. pylori* eradication and complicated urinary tract infections.

Studies, thus far, have also shown that finafloxacin retains additional various positive features of other marketed fluoroquinolones, including a good safety profile.

MATERIALS AND METHODS

**Bacterial strains**
Test strains:
A. anitratus (1), E. coli (8), E. cloacae (2), K. pneumoniae (2), P. vulgaris (1), P. rettgeri (1), P. stuartii (1), P. aeruginosa (3), S. aureus (13), S. haemolyticus (3), S. marcescens (2), S. saprophyticus (1), and *S. warneri* (1).

Quality control strains: E. coli ATCC® 25922, *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 29213.

**Reagents**
Finafloxacin powder (MerLion Pharmaceuticals Pte. Ltd, Singapore); Etest Finafloxacin (FIN) MIC range 0.002 – 32 µg/mL (AB bioMérieux, Solna, Sweden); Mueller Hinton agar and broth (BBL, Maryland, USA) at pH 5.8 and 7.2.

**Procedure**
Etest was used according to the manufacturer’s instructions and tested at both pH 5.8 and pH 7.2. AD and BMD were performed using the CLSI procedures and tested at both pH 5.8 and pH 7.2. The MIC was read at complete inhibition of growth.

RESULTS

**Table 1. Etest MIC for different species at acidic and neutral pH**

<table>
<thead>
<tr>
<th>Species</th>
<th>MIC range (µg/mL)</th>
<th>pH 5.8</th>
<th>pH 7.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. pylori</td>
<td>0.25 – 32</td>
<td>0.125</td>
<td>0.125</td>
</tr>
<tr>
<td>P. stuartii</td>
<td>0.047</td>
<td>0.032</td>
<td>0.032</td>
</tr>
<tr>
<td>P. vulgaris</td>
<td>0.125</td>
<td>0.064</td>
<td>0.064</td>
</tr>
<tr>
<td>S. marcescens</td>
<td>0.5 – 32</td>
<td>0.125</td>
<td>0.125</td>
</tr>
<tr>
<td>S. haemolyticus</td>
<td>0.008</td>
<td>0.023</td>
<td>0.032</td>
</tr>
<tr>
<td>S. saprophyticus</td>
<td>0.064</td>
<td>0.064</td>
<td>0.064</td>
</tr>
<tr>
<td>E. coli ATCC 25922</td>
<td>0.125</td>
<td>0.125</td>
<td>0.125</td>
</tr>
<tr>
<td>S. aureus ATCC 29213</td>
<td>0.032</td>
<td>0.032</td>
<td>0.032</td>
</tr>
<tr>
<td>P. aeruginosa ATCC 27853</td>
<td>0.5</td>
<td>0.125</td>
<td>0.125</td>
</tr>
</tbody>
</table>

MIC endpoints were generally clear-cut for most organisms. Macro- and microcolonies were occasionally seen in Etest FIN inhibition ellipses for a few strains, such colonies are not specific to finafloxacin. The MIC was read at complete inhibition of growth.

**Table 2. Comparison of MIC methods at acidic and neutral pH**

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Equation</th>
<th>R²</th>
<th>EA ± 1 dil.</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD vs. BMD, pH 5.8</td>
<td>y = 1.54x + 0.39</td>
<td>0.99</td>
<td>100</td>
</tr>
<tr>
<td>AD vs. BMD, pH 7.2</td>
<td>y = 1.77x + 0.33</td>
<td>0.98</td>
<td>100</td>
</tr>
<tr>
<td>AD vs. AD, pH 5.8</td>
<td>y = 1.22x + 0.27</td>
<td>0.98</td>
<td>100</td>
</tr>
<tr>
<td>AD vs. AD, pH 7.2</td>
<td>y = 1.25x + 0.20</td>
<td>0.98</td>
<td>100</td>
</tr>
<tr>
<td>BMD vs. BMD, pH 5.8</td>
<td>y = 1.28x + 0.25</td>
<td>0.98</td>
<td>100</td>
</tr>
<tr>
<td>BMD vs. BMD, pH 7.2</td>
<td>y = 1.30x + 0.20</td>
<td>0.98</td>
<td>100</td>
</tr>
</tbody>
</table>

EA = Essential Agreement

**Table 3. Quality control results and tentative QC ranges for Etest FIN (µg/mL)**

<table>
<thead>
<tr>
<th>Strain</th>
<th>pH 4.5</th>
<th>pH 5.0</th>
<th>pH 5.6</th>
<th>pH 6.0</th>
<th>pH 6.5</th>
<th>pH 7.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>K. coli ATCC 13882</td>
<td>0.032</td>
<td>0.047</td>
<td>0.064</td>
<td>0.080</td>
<td>0.125</td>
<td>0.168</td>
</tr>
<tr>
<td>S. aureus ATCC 29213</td>
<td>0.032</td>
<td>0.047</td>
<td>0.064</td>
<td>0.080</td>
<td>0.125</td>
<td>0.168</td>
</tr>
<tr>
<td>P. vulgaris ATCC 27853</td>
<td>0.032</td>
<td>0.047</td>
<td>0.064</td>
<td>0.080</td>
<td>0.125</td>
<td>0.168</td>
</tr>
</tbody>
</table>

**CONCLUSIONS**

- MIC testing of finafloxacin with Etest, agar and broth dilution reference methods provides substantially equivalent results at both neutral and acidic pH (EA ± 1 dilution 88-100 %), and demonstrates higher activity of FIN at slightly acidic pH.
- Etest agreement with CLSI methods was lower at pH 5.8 primarily due to Etest being more efficient in detecting the resistant subpopulations.
- Etest MIC values at pH 5.8 were approximately 2-4 dilutions lower than those at pH 7.2.
- Etest with a wide concentration range (15 dilutions) comprise a useful MIC tool for drug development studies with FIN and for future studies with *H. pylori*.

Illustrations of Etest Finafloxacin results at neutral and acidic pH

Figure 1. P. stuartii CDC 2083
Figure 2. S. aureus ATCC 29213
Figure 3. E. coli ECI 2119 FQR

Comparison of Methods for Finafloxacin MIC Testing at Acidic and Neutral pH
A. Engelhardt, A. Yusof, P. Ho, C. Johansson, K. Sjöström
AB bioMérieux, Dalvägen 10, Solna, Sweden
elenst@abbiomierux.se
MIC Testing of *Helicobacter pylori* using Etest® Finafloxacin and the Reference Agar Dilution Method

A. Engelhardt, A. Yusof, P. Ho, C. Johansson, K. Sjöström
AB bioMérieux Solna Sweden

**INTRODUCTION**

*Helicobacter pylori* (HP) is a Gram negative bacteria commonly found in the gastric mucosa, where it can reside without any clinical symptoms. However, this pathogen has been associated with e.g. peptic ulcers and gastric cancer.

Since the treatment of HP infections used today is complex and can lead to side effects and cross-resistance, it is of great importance to find the most suitable drug.

Finafloxacin hydrochloride (FIN) is a novel 8-cyano fluoroquinolone that exhibits optimal activity at slightly acidic conditions, where other fluoroquinolones lose activity. The intended use of FIN is for the therapy of bacterial infections such as HP infections, since the bacteria harbours in an acidic environment.

The Etest FIN gradient is preformed, predefined and stable which makes the system suitable for testing of fastidious organisms like HP with varying growth rates.

**MATERIAL AND METHODS**

**Strains**

Test isolates: A total of 36 *H. pylori* clinical isolates, including fluoroquinolone resistant strains, were tested in quadruplicate.

Quality control strain: *H. pylori* ATCC 43504 was tested in quintuplicate.

**Reagents**

Finafloxacin powder (MerLion Pharmaceuticals Pte. Ltd, Singapore); Mueller Hinton agar (BBL, Maryland, USA); Etest Finafloxacin (FIN) MIC range 0.002 – 32 µg/mL (AB bioMérieux, Solna, Sweden).

**Procedure**

Etest was used according to manufacturers instruction and agar dilution was performed according to CLSI guidelines. Both methods were read after 3 and 5 days of incubation.

**OBJECTIVES**

The aim of the study was to compare MIC testing with Etest Finafloxacin and the CLSI agar dilution reference method using clinical *Helicobacter pylori* isolates.

**RESULTS**

Etest FIN and CLSI agar dilution method were shown to be comparable, although the correlation between Etest and AD were slightly better after 3 days (table 1), i.e. the recommended incubation time. Resistant micro/macro subcolonies were seen in the inhibition ellipse for a few isolates when tested by Etest, especially after 5 days of incubation (figure 3A and 3B). These subcolonies are not specific to finafloxacin and the clinical relevance needs to be further investigated.

**CONCLUSIONS**

- Etest FIN vs. CLSI agar dilution MIC results showed good agreement after 3 and 5 days of incubation.
- Recommended incubation time of *Helicobacter pylori* is 3 days.
- Etest FIN was more efficient than AD in detecting resistant subpopulations.
- Etest is a useful tool for testing new agents against *Helicobacter pylori*.

**Table 1. Essential agreements Etest vs. agar dilution**

<table>
<thead>
<tr>
<th>Incubation time</th>
<th>% EA, modal MIC ± 1 dil</th>
<th>% EA, modal MIC ± 2 dil</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 days</td>
<td>83.3 ± 1 dil</td>
<td>94.4 ± 2 dil</td>
</tr>
<tr>
<td>5 days</td>
<td>77.8 ± 1 dil</td>
<td>94.4 ± 2 dil</td>
</tr>
</tbody>
</table>

**Table 2. Intralaboratory reproducibility of Etest and agar dilution**

<table>
<thead>
<tr>
<th>Incubation time</th>
<th>% Reproducibility, modal MIC ± 1 dil</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 days</td>
<td>93.1 ± 1 dil</td>
</tr>
<tr>
<td>5 days</td>
<td>90.3 ± 1 dil</td>
</tr>
</tbody>
</table>

**Table 3. Tentative quality control ranges for Etest Finafloxacin (µg/mL)**

<table>
<thead>
<tr>
<th>Organism</th>
<th>3 and 5 days incubation</th>
<th>Tentative Etest QC range</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>H. pylori</em> ATCC 43504</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AD (n=30)</td>
<td>0.125 - 0.25</td>
<td>0.064 - 0.25</td>
</tr>
<tr>
<td>Etest (n=20)</td>
<td></td>
<td>Mode: 0.125</td>
</tr>
<tr>
<td></td>
<td>0.064 - 0.19</td>
<td>Mode: 0.125</td>
</tr>
</tbody>
</table>

**Figure 1. Etest FIN vs. AD; 3 days**

**Figure 2. Etest FIN vs. AD; 5 days**

**Figure 3. Illustrations of Etest Finafloxacin results**

A - *H. pylori*  
B - *H. pylori* ATCC 43504
Comparative Inhibitory and Bactericidal Activities of Finafloxacin and Ciprofloxacin against Gram-Negative and Gram-Positive UTI-pathogens under Physiological Conditions and at Varying pH-values

S. SCHUBERT, W. STUBBINGS, A. DALHOFF

Introduction

Finafloxacin (FIN, Figure 1) is a novel broad spectrum fluoroquinolone (FQ) belonging to a new 8-cyano subclass which exhibits improved in vitro activity at slightly acidic pH and is therefore intended for treatment of UTI. The antibacterial and bactericidal activities of FIN and CIP were compared in artificial urine medium which reflects the physiological conditions of pH, ionic strength and chemical composition, encountered in vivo.

Methods

Comparative inhibitory and bactericidal activities of FIN and CIP were determined in artificial urine medium which reflects the physiological conditions of pH, ionic strength and chemical composition, encountered in vivo.

The MICs of FIN and CIP were determined against 34 strains (E. coli, K. pneumoniae, Enterobacteriaceae, P. aeruginosa incl. CIP-resistant and ESBL producers) using CLSI methodology in cation adjusted Mueller-Hinton broth (CAMHB) at pH 7.2 and 5.8 and in artificial urine (pH 5.8), and the strain used for the study was: 35 strains of Gram-negative and Gram-positive bacteria were tested; these included a number with resistance determinants.

Conclusions

FIN was more active in CAMHB at all pHs (5.8) than at pH 7.2, unlike CIP, which had reduced activity at an acidic pH.

• These bacteriostatic (MICs) and bactericidal activities (time kill curves) of FIN also differ favourably from those of CIP under conditions mimicking UTIs. The activity of FIN in artificial urine was both quantitative and qualitatively different from that of CIP. These findings indicate that FIN may be effective in the treatment of UTIs.

<table>
<thead>
<tr>
<th>Literature</th>
</tr>
</thead>
</table>

Figure 1. Finafloxacin hydrobromide.
Bactericidal Activity Of Finafloxacin Against Difficult To Kill Growth Forms of Escherichia coli

C. Y. GOH1, F. GOH1, W. STUBBINGS1, H. P. KROLL2, H. LABISCHINSKI1;
1Merlion Pharmaceuticals Pte Ltd, Singapore, Singapore, 2Bayer HealthCare AG, Elberfeld, Germany.

Revised Abstract

Methods

Membrane filter model

E. coli C600 were inoculated onto 0-45 µm filter cartridges under a continuous flow of brain heart infusion broth (BHI), pH 6.2. Once steady state had been established (10^8 – 10^9 CFU/cm2 of periwinkle), FIN, ciprofloxacin (CIP), levofloxacin (LVX) or moxifloxacin (MXF) (all 5 mg/L) were perfused for 24 h, followed by 1 h of drug-free medium. Representative subpopulations (titanium frequencies) were defined as the fraction of viable cells that were recovered following exposure of high cell densities of E. coli adherent populations to biocides and subsequently regrown in drug-free medium. The comparative bactericidal activity of FIN, CIP, LVX or MXF was determined against E. coli and S. aureus adherent cultures. Bacterial populations were exposed to FIN (10 mg/L), CIP (10 mg/L), LVX (5 mg/L) and MXF (5 mg/L) for 24 h and the surviving fractions were determined by plating on drug-free medium.

Results and Discussion

Results: FIN exhibited superior killing to CIP, LVX and MXF against filter membrane-adherent E. coli. FIN was faster and FIN was the only drug to prevent the treated population from regrowing in drug-free media.

Catheter-adherent populations

Catheter-adherent populations of E. coli and S. aureus exhibited age-dependent susceptibilities to antibiotics. For example at 3 days, adherent populations of both species were completely eradicated from the catheter lumen exposure to 0.1 mg/L of FIN or CIP. Older populations began to exhibit phenotypic resistance to these drugs and hence were more difficult to treat. FIN exhibited superior bactericidal activity to CIP at concentrations of 1 mg/L, and above against 4- and 6-day old catheter adherent populations of E. coli and S. aureus (Figure 4). On average, FIN reduced such populations to 1 to 2 log10(CFU) lower than equivalent CIP treated populations.

Conclusions

• FIN exhibited superior killing to CIP, LVX and MXF against filter membrane-adherent E. coli. Killing was faster and FIN was the only drug to prevent the treated population from re-growing in drug-free media.

• FIN also exhibited superior bactericidal activity to CIP against catheter adherent E. coli and S. aureus. Killing was superior in terms of the lower numbers of surviving cells.

• Exposure of stationary-phase E. coli to FIN also resulted in a more extensive killing than CIP and LVX.

• These findings show that FIN is superior to other FQs in terms of the speed and extent of its bactericidal activity against non-growing and adherent E. coli.

Literature

Selection and Characterisation of Finafloxacin, Ciprofloxacin and Levofloxacin Resistant Mutants of Escherichia coli

P. LEOW1, W. STUBBINGS1, W. LABISCHINSKI2
1MerLion Pharmaceuticals Pte Ltd, Singapore, 2MerLion Pharmaceuticals GmbH, Berlin, Germany.

Abstract

Finafloxacin (FIN) is novel fluoroquinolone (FQ) belonging to a new 8-oxo-8-carboxylic subfamilies. FIN exhibits optimal efficacy at slightly acidic pH (5.0 – 6.0), under which other FQs show decreased activity. Therefore, FIN is intended for therapeutic use against bacterial infections associated with acidic environments, as the virulence and systemic mechanisms of drug resistance to ciprofloxacin (CIP) and levofloxacin (LVX) was investigated in E. coli at pH 7.2 and pH 5.8.

Methods

Minimum inhibitory concentrations (MICs) were determined according to the CLSI procedure for broth microdilution at pH 7.2 and pH 5.8. Target genes mutation in gene segments of gyrA and parC of CIP, LVX and FIN resistant mutants were sequenced from PCR products. DNA sequences of mutants were aligned with parent.

Results

Resistance frequencies (5 x MIC) of first step mutants to FIN, CIP and LVX were 4.1 x 10^-10, 2.2 x 10^-10 and 1.3 x 10^-10 respectively. First step mutants exhibited an ~32-fold decrease in susceptibility over the parent and a relative decrease in susceptibility to the comparator FQs. All first step mutants (FIN, CIP & LVX) developed mutations within the quinolone resistance determination region (QRDR) of gyrA, followed by substitutions G81D, S83L and D87N. No mutations in the QRDR of parC were detected.

Conclusion

FIN mutants arose at similar frequencies to the CIP and LVX mutants and exhibited similar mutations in susceptibility suggesting that FIN has the same low potential for resistance development. Mutations within the QRDR of gyrA were identified in FIN and LVX first-step mutants of E. coli indicating this as a primary target.

Results and Discussion

The potential for resistance development to FIN, CIP and LVX in E. coli was investigated through comparison of first-step spontaneous resistance frequencies (Figure 2) and mutation prevention concentrations (Figure 3) at pH 7.2 and pH 5.8. At pH 7.2, the MICs for FIN, CIP and LVX were 0.008, 0.004 and 0.001 mg/L respectively. At pH 7.2, the MICs for FIN, CIP and LVX were 0.125, 0.063 and 0.016 mg/L respectively.

The potential for resistance development to FIN, CIP and LVX was also compared by serial passage of E. coli in the presence of subinhibitory FQ concentrations. The concentration of FIN at which the passaged culture could grow steadily increased over 22 passages, to 7.3 – 32 times greater than the starting concentration (Figure 4).

Single-step, agar selected, FIN, CIP and LVX mutants are listed in Table 1. Mutants to each drug exhibited a similar susceptibility profile, comprising of a 32 – 32-fold increase in MIC to each of the FQs tested. Mutants to each drug harbored single point mutations conferring one of the following amino acid substitutions within the QRDR of gyrA: G81D, S83L and D87N. No mutations were detected in the QRDR of parC.

Conclusions

• FIN exhibited improved antibacterial activity at low pH whereas CIP and LVX (along with other marketed FQs) lose activity. The mutant selection window for these FQs changed accordingly (and relative to potency) with pH.

• Resistance frequencies for FIN in E. coli were comparable to those for CIP and LVX at 8 x MIC and at all 8 x MIC.

• Single-step mutants to FIN, CIP and LVX exhibited relative decreases in susceptibility and common target allelic profiles, indicating gyrA as the primary target.

These findings imply that FIN would have an advantage, in terms of its mutant selection window, under conditions of low pH, reflecting its unusual pH activation profile.

Literature

Pharmacokinetics (PK) and In Vivo Efficacy of Oral Finafloxacin (FIN) and Comparators in Rodent Models of Systemic Infections

R. Endemann¹, C. Ladel¹, W. Stubbings², H. Labischinski²
Bayer HealthCare AG, Elberfeld, Germany, MerLion Pharmaceuticals Pte Ltd, Singapore.

Results and Discussion

Pharmacokinetics: Serum concentrations were measured after oral dosing with FIN, MXF, CIP or LVX by bioassay. Blood samples were collected from 3 mice/time point, were prepared and the concentrations measured by zone diffusion bioassay against E. coli or S. aureus. AUC, Cmax, and T1/2 values were calculated.

Mouse infections: Overnight cultures of each microorganism were diluted and recultured so that bacteria were in the early logarithmic phase of growth. Female C57BL/6 mice, 18-20g body weight were infected intraperitoneally (i.p.) with a bacterial suspension in physiological saline or 5% mucin in saline. An inoculum exceeding the LD50 was used.

Entercoccus faecalis infection – Groups of 6 mice were infected i.p. with 2.4 x 10^8 CFU/ml of strain 27159 in 5% mucin. Treatment was by the oral route or by the intravenous (i.v.) route at 30 min post infection with 1, 10, 25 mg/kg of FIN, MXF, CIP, LVX. The survival at 5 days post infection was plotted.

Moraxella catarrhalis infection – Groups of 6 mice were infected i.p. with 1.3 x 10^8 CFU/ml in 5% mucin. Treatment was by the oral route at 30 min post infection with 1, 10, 25 mg/kg of FIN, MXF, CIP or LVX. On day 1 post infection mice were killed, lungs removed and homogenised (PORTER 5 Homogenizer) in sterile saline. Viable bacteria were determined by plating serial 10-fold dilutions of the homogenates in duplicate on agar plates. The colony forming units (CFUs) were counted after overnight incubation.

Escherichia coli infection - Groups of 6 mice were infected i.p. with 3.2 x 10^7 CFU/ml mouse of strain DSM 10650. Treatment was by the oral route at 30 min post infection with 0.1, 0.5, 1.0, or 10 mg/kg of FIN, MXF, CIP or LVX. The survival at 5 days post infection was plotted.

Results and Discussion

Table 1. Oral mouse PK values (normalised to 1 mg/kg).

<table>
<thead>
<tr>
<th>Compound</th>
<th>AUC (µg/ml*hour)</th>
<th>Cmax (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIN</td>
<td>0.271</td>
<td>0.264</td>
</tr>
<tr>
<td>MXF</td>
<td>0.153</td>
<td>0.172</td>
</tr>
<tr>
<td>LVX</td>
<td>0.104</td>
<td>0.035</td>
</tr>
<tr>
<td>CIP</td>
<td>0.220</td>
<td>0.182</td>
</tr>
</tbody>
</table>

Additionaly, FIN displayed an excellent safety profile in a wide range of preclinical, in vivo, toxicity assays [4] and was well tolerated in healthy volunteers [5]. These attributes suggest that FIN warrants clinical investigation for bacterial infections that are usually associated with low pH such as urinary tract infection and Helicobacter pylori eradication.

FIN displayed favorable pharmacokinetic parameters in mice, when compared alongside several best in class FQs, ciprofloxacin (CIP), levofloxacin (LVX) and moxifloxacin (MXF). The therapeutic potential of FIN was then assessed in a series of rodent bacteremia models.

Introduction

Finafloxacin (FIN, Figure 1) is a novel, broad spectrum fluoroquinolone (FQ) that belongs to a new 8-cyano subclass [1]. FIN contains a novel chiral base component which confers improved antibacterial activity at slightly acidic pH (5.0 – 6.0) under which other marketed FQs exhibit significantly reduced activity [2]. FIN also exhibited superior activity against adherent bacteria in vitro [3].

Additionally, FIN displayed an excellent safety profile in a wide range of preclinical, in vivo, toxicity assays [4] and was well tolerated in healthy volunteers [5]. These attributes suggest that FIN warrants clinical investigation for bacterial infections that are usually associated with low pH such as urinary tract infection and Helicobacter pylori eradication.

FIN displayed favorable pharmacokinetic parameters in mice, when compared alongside several best in class FQs, ciprofloxacin (CIP), levofloxacin (LVX) and moxifloxacin (MXF). The therapeutic potential of FIN was then assessed in a series of rodent bacteremia models.

Figure 1. Finafloxacin hydrochloride.

Pharmacokinetics (PK) and In Vivo Efficacy of Oral Finafloxacin (FIN) and Comparators in Rodent Models of Systemic Infections

Methods

Pharmacokinetics: Serum concentrations were measured after oral dosing with FIN, MXF, CIP or LVX by bioassay. Blood samples were collected from 3 mice/time point, were prepared and the concentrations measured by zone diffusion bioassay against E. coli or S. aureus. AUC, Cmax, and T1/2 values were calculated.

Mouse infections: Overnight cultures of each microorganism were diluted and recultured so that bacteria were in the early logarithmic phase of growth. Female C57BL/6 mice, 18-20g body weight were infected intraperitoneally (i.p.) with a bacterial suspension in physiological saline or 5% mucin in saline. An inoculum exceeding the LD50 was used.

Entercoccus faecalis infection – Groups of 6 mice were infected i.p. with 2.4 x 10^8 CFU/ml of strain 27159 in 5% mucin. Treatment was by the oral route or by the intravenous (i.v.) route at 30 min post infection with 1, 10, 25 mg/kg of FIN, MXF, CIP, LVX. The survival at 5 days post infection was plotted.

Moraxella catarrhalis infection – Groups of 6 mice were infected i.p. with 1.3 x 10^8 CFU/ml in 5% mucin. Treatment was by the oral route at 30 min post infection with 1, 10, 25 mg/kg of FIN, MXF, CIP or LVX. On day 1 post infection mice were killed, lungs removed and homogenised (PORTER 5 Homogenizer) in sterile saline. Viable bacteria were determined by plating serial 10-fold dilutions of the homogenates in duplicate on agar plates. The colony forming units (CFUs) were counted after overnight incubation.

Escherichia coli infection - Groups of 6 mice were infected i.p. with 3.2 x 10^7 CFU/ml mouse of strain DSM 10650. Treatment was by the oral route at 30 min post infection with 0.1, 0.5, 1.0, or 10 mg/kg of FIN, MXF, CIP or LVX. The survival at 5 days post infection was plotted.

Conclusions:

FIN showed a greater reduction in the numbers of organisms surviving in the lungs of infected mice at all three dose levels. It was also more active in vitro than the three comparator FQs (figure 6).

Conclusions

FIN, a new, 8-cyano, broad spectrum FQ has activity in vivo against a wide range of pathogenic microorganisms in rodent bacteremia models.

The results shown here illustrate its superior activity compared with the comparator FQs MXF, CIP and LVX when administered by the oral route in mouse models of systemic infections caused by E. coli and E. faecalis. It was also more active than the other FQs against M. catarrhalis, reducing the numbers of organisms in the lungs of infected mice even at low doses.

These findings, taken together with the good broad spectrum activity in vitro against a number of important pathogenic species, including these resistant to other agents, the excellent tolerance seen by the oral route in Phase I studies in man and the lack of toxicity seen in preclinical in vivo toxicity tests, indicate that FIN is an excellent candidate for progression to the clinic.

References

In Vivo Efficacy of Finafloxicin in Difficult to Treat Animal Models of Infection

R. Endermann1, C. Ladeli, W. Stubblings2, H. Labischinski2; Bayer HealthCare AG, Elberfeld, Germany; 1MerLion Pharmaceuticals Pte Ltd, Singapore, Singapore.

Abstract

Background: Finafloxicin (FIN) is a novel fluoroquinolone (FQ) belonging to a new 8-cyano subclass that exhibits optimal efficacy at slightly acidic pH (5.0 – 6.0), under which other FQs show decreased activity. FIN was evaluated along with ciprofloxacin (CIP), levofloxacin (LEV) and moxifloxacin (MOX), in a wide range of in vivo models.

Methods: Female C57BL/6 mice (n = 6) were used. Bacterial inocula were administered by intraperitoneal injection. Infections were initiated, replication of cultured bacterial material or direct injection into the kidney, bladder, thigh, gastric pouch or abscesses. Treatment was commenced 0.5 h post-infection. End points were determined by % survival (at 7 days) or by reduction of bacterial counts (CFU/mL) in homogenized tissue.

Results: FIN (5 mg/kg) exhibited greater killing of S. aureus in the thigh muscle (10 mg/kg), E. coli and P. aeruginosa (5 mg/kg) when compared to CIP (10 mg/kg), LEV (5 mg/kg) and MOX (10 mg/kg). The colony forming units (CFU/mL) at 48 h in a S. aureus culture. The catheter was inserted and then implanted i.c. in mice. Treatment started 3 h later and continued IID until the day prior to removal. The moxifloxacin (UBA), diluted and plated to determine the CFU remaining. Infected abscess model – S. aureus and P. aeruginosa. Gelfoam was cut into pieces 1 x 1 cm and incubated overnight in sterile PBS, pH 7.4. The following day these were implanted i.c. on the back of mice. Within days a capsule formed around the implant and this was injected with 1 x 106 S. aureus or 4 x 105 P. aeruginosa. Treatment was with 10 mg/kg 2 h post infection.

Postoperative polymicrobial sepsis. Cecal ligation and puncture model mice were anaesthetised and the peritoneum opened with a small cut. The cecum was moved out of the peritoneum. The cecum was ligated and punctured with a 21G needle. The ligated intestine was replaced and the wound closed. 3 days of 10 mg/kg were given 4, 8 and 12 h post-infection. Efficacy was determined by survival over 7 days.

E. coli pyelonephritis. Mice were anaesthetised, the right flank shaved to expose the kidney. Holding the kidney before the skin, 10 µL of a suspension of E. coli (10 mg/kg) were injected i.c. directly into the kidney by using a 21G needle. Mice were treated with a single dose of 1 x 106 CFU 2 h post infection. Kidneys were removed 2 days later, homogenised and viable counts performed on dilutions of the homogenates.

Results and Discussion

S. aureus DSM 11823 infected thigh model. The effect of the FQs in reducing the numbers of staphylococcus in the thigh tissues is shown in Figure 2. FIN produced a dramatic fall of 5 log10 CFU recovered from the thigh homogenates at 10 mg/kg, far more than was seen with the other compounds. FIN and MOX had the lowest MICs (0.125 mg/L) but MOX, although being more active than CIP and LEV, was less active at all dose levels than FIN.

Figure 2. S. aureus infected thigh model – CFU reduction in thigh muscle.

Implanted Foreign Body Model S. aureus DSM 11823 infected catheter. The effect of the FQs in reducing the numbers of staphylococci in the catheters at 7 days post-implantation is shown in Figure 3. As in the thigh lesion model, FIN was the most active compound; reducing the numbers by > 6 logs. CIP had little or no effect and had the poorest MIC (0.5 mg/L). MOX was also effective but was still inferior to FIN. LVX had an intermediate effect.

Figure 3. S. aureus infected catheter – CFU reduction in catheter.

Conclusions

FIN had excellent activity in a range of infection models in mice chosen to reflect those that are difficult to treat in the clinic such as peritonitis (with remote organ failure), catheter colonisation and SSTI.

In general, the efficacy of FIN was superior to that of CIP, LVX and MOX.

The efficacy of FIN was better than expected from its MIC at pH 7.2, it was especially true in models of serious infection such as peritonitis, pyelonephritis, abscess and thigh muscle infection and may reflect the improved activity of FIN at an acidic pH.

These findings taken in conjunction with the excellent tolerance seen by the oral route in Phase I studies in man and the lack of toxicity seen in predictive in vivo toxicity tests, indicate that FIN is an excellent candidate for progression to the clinic.

Literature

Comparative Activity Between Finafloxacin (FIN) and Other Fluoroquinolones Against Bacterial and Eukaryotic Type II Topoisomerases

M. T. MULLER1, W. STUBBINGS2 A. VENTE2
1Topogen Inc, Port Orange, FL, 2MerLion Pharmaceuticals GmbH, Berlin, Germany.

Abstract

Finafloxacin (FIN, ciprofloxacin (CIP), moxifloxacin (MXF), clarithromycin (CLX) and enoxacin (ENX) and the topoisomerase poison VP16 were titrated against

Human Topoisomerase II: Human topo II isoform was added to 250 ng plated DNA Substrate for 30min at 37°C. The reactions were terminated with SDS (1%) and digested with proteinase K (50 µg/mL) for 30 min at 56°C and run on 1% agarose gels. Inhibition of the human enzyme was quantified as a function of released single stranded DNA.

Escherichia coli DNA Gyrase and DNA Topoisomerase IV: Supercoiled pHODT was released using human topo I to form open circular DNA. Addition of functional E. coli DNA gyrase or topoisomerase IV converted this substrate to supercoiled DNA. Inhibition of gyrase or topoisomerase activity was measured by quantification of released, linear DNA.

Comparative Activity Between Finafloxacin (FIN) and Other Fluoroquinolones Against Bacterial and Eukaryotic Type II Topoisomerases

Finafloxacin (FIN, ciprofloxacin (CIP), moxifloxacin (MXF), clarithromycin (CLX) and enoxacin (ENX) and the topoisomerase poison VP16 were titrated against

Human Topoisomerase II: Human topo II isoform was added to 250 ng plated DNA Substrate for 30min at 37°C. The reactions were terminated with SDS (1%) and digested with proteinase K (50 µg/mL) for 30 min at 56°C and run on 1% agarose gels. Inhibition of the human enzyme was quantified as a function of released single stranded DNA.

Escherichia coli DNA Gyrase and DNA Topoisomerase IV: Supercoiled pHODT was released using human topo I to form open circular DNA. Addition of functional E. coli DNA gyrase or topoisomerase IV converted this substrate to supercoiled DNA. Inhibition of gyrase or topoisomerase activity was measured by quantification of released, linear DNA.

Activity of FQs compared to the topoisomerase poison VP16 against human Topoisomerase II.

The human topo II isoform was sensitive to VP16 (CDL = 1 µg/mL, CL50 = 25 µg/mL). The activities (CDL and CL50) of the test compounds were determined against the human enzyme for the calculation of their relative selectivity indices [activity of test FQ (CDL or CL50) / activity of VP16 (CDL or CL50)].

Of these, ENX exhibited the highest selectivity index (based on CL50, 10, based on CDL, 50 against the eukaryotic enzyme. This was followed by CIP (R, 250) then FIN (100, 250) and MXF (50 - estimated). ENX exhibited the greatest selectivity index of the test compounds (both CDL and CL50 were undetectable). These data are summarized in Table 1 and Figure 2.

The CDL values of the FQs against human topo II indicate a range of selectivity indices spanning 50-500-fold. FIN exhibited an index of 250 which placed it among the other FQs in terms of its ratio of selectivity.

Activity of FQs against E. coli DNA gyrase (Table 2, Figure 3).

E. coli DNA gyrase activity in the presence of supercoiled DNA was inhibited by all test FQs in a concentration dependent manner. This inhibition was measured by quantification of released, linear DNA.

Results and Discussion

Activity of FQs compared to the topoisomerase poison VP16 against human Topoisomerase II.

The human topo II isoform was sensitive to VP16 (CDL = 1 µg/mL, CL50 = 25 µg/mL). The activities (CDL and CL50) of the test compounds were determined against the human enzyme for the calculation of their relative selectivity indices [activity of test FQ (CDL or CL50) / activity of VP16 (CDL or CL50)].

Of these, ENX exhibited the highest selectivity index (based on CL50, 10, based on CDL, 50 against the eukaryotic enzyme. This was followed by CIP (R, 250) then FIN (100, 250) and MXF (50 - estimated). ENX exhibited the greatest selectivity index of the test compounds (both CDL and CL50 were undetectable). These data are summarized in Table 1 and Figure 2.

The CDL values of the FQs against human topo II indicate a range of selectivity indices spanning 50-500-fold. FIN exhibited an index of 250 which placed it among the other FQs in terms of its ratio of selectivity.

Activity of FQs against E. coli DNA gyrase (Table 2, Figure 3).

E. coli DNA gyrase activity in the presence of supercoiled DNA was inhibited by all test FQs in a concentration dependent manner. This inhibition was measured by quantification of released, linear DNA.

Results and Discussion

Activity of FQs compared to the topoisomerase poison VP16 against human Topoisomerase II.

The human topo II isoform was sensitive to VP16 (CDL = 1 µg/mL, CL50 = 25 µg/mL). The activities (CDL and CL50) of the test compounds were determined against the human enzyme for the calculation of their relative selectivity indices [activity of test FQ (CDL or CL50) / activity of VP16 (CDL or CL50)].

Of these, ENX exhibited the highest selectivity index (based on CL50, 10, based on CDL, 50 against the eukaryotic enzyme. This was followed by CIP (R, 250) then FIN (100, 250) and MXF (50 - estimated). ENX exhibited the greatest selectivity index of the test compounds (both CDL and CL50 were undetectable). These data are summarized in Table 1 and Figure 2.

The CDL values of the FQs against human topo II indicate a range of selectivity indices spanning 50-500-fold. FIN exhibited an index of 250 which placed it among the other FQs in terms of its ratio of selectivity.

Activity of FQs against E. coli DNA gyrase (Table 2, Figure 3).

E. coli DNA gyrase activity in the presence of supercoiled DNA was inhibited by all test FQs in a concentration dependent manner. This inhibition was measured by quantification of released, linear DNA.

Results and Discussion

Activity of FQs compared to the topoisomerase poison VP16 against human Topoisomerase II.

The human topo II isoform was sensitive to VP16 (CDL = 1 µg/mL, CL50 = 25 µg/mL). The activities (CDL and CL50) of the test compounds were determined against the human enzyme for the calculation of their relative selectivity indices [activity of test FQ (CDL or CL50) / activity of VP16 (CDL or CL50)].

Of these, ENX exhibited the highest selectivity index (based on CL50, 10, based on CDL, 50 against the eukaryotic enzyme. This was followed by CIP (R, 250) then FIN (100, 250) and MXF (50 - estimated). ENX exhibited the greatest selectivity index of the test compounds (both CDL and CL50 were undetectable). These data are summarized in Table 1 and Figure 2.

The CDL values of the FQs against human topo II indicate a range of selectivity indices spanning 50-500-fold. FIN exhibited an index of 250 which placed it among the other FQs in terms of its ratio of selectivity.

Results and Discussion

Activity of FQs compared to the topoisomerase poison VP16 against human Topoisomerase II.

The human topo II isoform was sensitive to VP16 (CDL = 1 µg/mL, CL50 = 25 µg/mL). The activities (CDL and CL50) of the test compounds were determined against the human enzyme for the calculation of their relative selectivity indices [activity of test FQ (CDL or CL50) / activity of VP16 (CDL or CL50)].

Of these, ENX exhibited the highest selectivity index (based on CL50, 10, based on CDL, 50 against the eukaryotic enzyme. This was followed by CIP (R, 250) then FIN (100, 250) and MXF (50 - estimated). ENX exhibited the greatest selectivity index of the test compounds (both CDL and CL50 were undetectable). These data are summarized in Table 1 and Figure 2.

The CDL values of the FQs against human topo II indicate a range of selectivity indices spanning 50-500-fold. FIN exhibited an index of 250 which placed it among the other FQs in terms of its ratio of selectivity.

Results and Discussion

Activity of FQs compared to the topoisomerase poison VP16 against human Topoisomerase II.

The human topo II isoform was sensitive to VP16 (CDL = 1 µg/mL, CL50 = 25 µg/mL). The activities (CDL and CL50) of the test compounds were determined against the human enzyme for the calculation of their relative selectivity indices [activity of test FQ (CDL or CL50) / activity of VP16 (CDL or CL50)].

Of these, ENX exhibited the highest selectivity index (based on CL50, 10, based on CDL, 50 against the eukaryotic enzyme. This was followed by CIP (R, 250) then FIN (100, 250) and MXF (50 - estimated). ENX exhibited the greatest selectivity index of the test compounds (both CDL and CL50 were undetectable). These data are summarized in Table 1 and Figure 2.

The CDL values of the FQs against human topo II indicate a range of selectivity indices spanning 50-500-fold. FIN exhibited an index of 250 which placed it among the other FQs in terms of its ratio of selectivity.

Results and Discussion

Activity of FQs compared to the topoisomerase poison VP16 against human Topoisomerase II.

The human topo II isoform was sensitive to VP16 (CDL = 1 µg/mL, CL50 = 25 µg/mL). The activities (CDL and CL50) of the test compounds were determined against the human enzyme for the calculation of their relative selectivity indices [activity of test FQ (CDL or CL50) / activity of VP16 (CDL or CL50)].

Of these, ENX exhibited the highest selectivity index (based on CL50, 10, based on CDL, 50 against the eukaryotic enzyme. This was followed by CIP (R, 250) then FIN (100, 250) and MXF (50 - estimated). ENX exhibited the greatest selectivity index of the test compounds (both CDL and CL50 were undetectable). These data are summarized in Table 1 and Figure 2.

The CDL values of the FQs against human topo II indicate a range of selectivity indices spanning 50-500-fold. FIN exhibited an index of 250 which placed it among the other FQs in terms of its ratio of selectivity.
**Introduction**

Finafloxacin (FIN, Figure 1) is a novel, broad spectrum fluoroquinolone (FQ) that belongs to a new 8-azaquinolone family (8). FIN contains a novel chiral base component which confers improved antibacterial activity at slightly acidic pH (pH 5.0 – 6.0) under which other marketed FQs exhibit significantly reduced activity.

FIN exhibits superior activity compared with comparator FQs against adherent bacteria in vitro and in a wide range of in vitro and in vivo infection models (4, 5). These findings suggest that FIN warrants clinical investigation for bacterial infections that are usually associated with low pH such as urinary tract infection and Helicobacter pylori eradication.

Several novel FQs have failed at late stages of development or have been withdrawn due to concerns over safety / toxicology. Therefore toxicological profiling of FIN was addressed during early stages of development in an extensive set of predoxic, in vivo toxicity assays.

**Methods**

Mouse cytotoxicity – A permanent mouse macrophage cell line (HT-29/AR) obtained from ATCC was incubated with compounds for 72 h in DMEM in microtitre plates. After washing, cell viability was determined using a neutral red assay.

Excitatory and neurotoxic potential – Brain slices from adult Sprague-Dawley rats were used. Following incubation with compounds, the slice preparation was mounted vertically in a recording chamber and superfused. Changes in excitatory postsynaptic potentials (EPSPs) were monitored at 30–60 min.

Phototoxicity – Mouse fibroblasts and a NOECA of 30 µg/mL. FINA was classified as non-phototoxic in this system with a NOECA of 10 (dog) and 30 µg/mL (human).

**Results and Discussion**

Primary human and dog chondrocytes. FIN showed no toxicity in any of the tests used, resulting in a NOECA of 100 µg/mL. These are summarised in Figure 3. In contrast, CIP had a NOECA of 10 (dog) and 30 µg/mL (human).

**Conclusions**

• FINA showed a number of toxic effects, some of which had to be withdrawn. The tests systems used here are all well validated in vitro and in vivo systems designed to test possible toxic effects.

• Juvenile dogs have been shown to be more susceptible than rodents to which FINA is associated with FQ-induced arthropathy. CP-DL displayed toxicity and potential to the dog in this test ( unlike SPA).

• Compounds inhibiting the ERG channel have been shown to prolong the cardiac action potential and hence the QT interval in man. FIN had no effect in this test ( unlike SPA).

• These findings (summarised in Figure 6) taken in conjunction with the excellent tolerability by the oral route in Phase I studies in man and the good activity in animal infection models (4, 5) indicate that finafloxacin is an excellent candidate for progression to the clinic.

**References**


**Authors**

G. Schmuck 1, G. Wasinski-Kempka 1, A. Vente 2, H. Labischinski 2

1 Bayer HealthCare AG, Wuppertal, Germany; 2 MerLion Pharmaceuticals GmbH, Berlin, Germany.

**Contact Information**

Dr. Andrea Vente
MerLion Pharmaceuticals GmbH
Robert-Riise-Str. 10
13127 Berlin
Phone: +49(0)33495-4037
vente@merlionpharma.de
A Phase I Study to Determine Safety, Tolerability and Pharmacokinetics (PK) of Finafloxacin (FIN) in Healthy Subjects

H. PATEL1, A. Andresen1, H-D. Heillmann1, M. Seierling1, L. Lopez2, R. Pokorny2, H. Labischinski1

1MerLion Pharmaceuticals GmbH, Berlin, Germany, 2Swiss Pharma Contract, Basel, Switzerland

Methods

The study was an inpatient, randomized, double-blind, placebo-controlled, dose-escalation study to evaluate the safety, tolerability and pharmacokinetic profiles of single and multiple doses of finafloxacin hydrochloride administered orally to healthy male and female subjects, aged between 18 and 55 years. Subjects received single doses of 25, 50, 100, 200, 400 or 800 mg. For the multiple dose study, subjects received 7 daily doses of 150, 300, 600 or 800 mg.

Efficacy measures were made from the time of dosing and up to 4 h post-dosing and at 8 h and 24 h. Blood and urine samples were collected prior to the study, at entry, at all the end of evaluation for clinical chemistry, haematology and urinalysis. Plasma and urine samples were collected at various intervals from previous 48 h post dose for pharmacokinetic analysis. Urinary bacteriological activity was determined for 250 and 800 mg single dose. See poster F1-2042 for further details.

FIN concentrations were estimated in plasma and urine samples by a validated LC/MS/MS method. The lower quantification limit was 5 ng/ml in plasma and 100 ng/ml in urine.

Pharmacokinetic parameters were evaluated using non-compartmental analysis. PK parameters, urinary recovery and renal clearance were determined.

All adverse events were reported and assessed as mild, moderate or severe.

Safety & Tolerability:

FIN was well tolerated following single dose and when given for seven days at a range of doses up to 800 mg. Human safety data do not suggest any quantitatively higher or qualitatively different toxicity for FIN as compared with placebo.

Overall, these findings indicate that the risk of serious adverse reactions to finafloxacin hydrochloride can be expected to be very low. Given the possible therapeutic effects of FIN, further clinical development of the drug appears justified and can be recommended.

Results and Discussion

FIN absorption was rapid, with Cmax values of 0.5 to 2.0 h. AUCmax increased almost linearly with dose. The median value of total oral body clearance was 29.0 and 35.8 L/hr for 400 mg and 800 mg, respectively.

Urinary pharmacokinetics for single dose:

Response rates are consistent with previous IV IV post dose for pharmacokinetic analysis. Urinary bacteriological activity was determined for 250 and 800 mg single dose. See poster F1-2042 for further details.

FIN concentrations were estimated in plasma and urine samples by a validated LC/MS/MS method. The lower quantification limit was 5 ng/ml in plasma and 100 ng/ml in urine.

Pharmacokinetic parameters were evaluated using non-compartmental analysis. PK parameters, urinary recovery and renal clearance were determined.

Adverse events were reported and assessed as mild, moderate or severe.

Safety & Tolerability:

FIN was well tolerated following single dose and given for seven days at a range of doses up to 800 mg. Human safety data do not suggest any quantitatively higher or qualitatively different toxicity for FIN as compared with placebo.

Overall, these findings indicate that the risk of serious adverse reactions to finafloxacin hydrochloride can be expected to be very low. Given the possible therapeutic effects of FIN, further clinical development of the drug appears justified and can be recommended.

References


Figure 1. Finafloxacin hydrochloride.

Figure 2. Plasma concentration vs. time profile of escalating single dose in healthy subjects. The mean (median) maximum concentration of FIN in urine was 120 (85.2) mg/l at 2 to 4 hours following 400 mg dose and 150 (127) mg/l at 4 to 8 hours following 800 mg dose. The median value of renal clearance was 7.5 and 11 L/h following 400 mg and 800 mg, respectively.

Plasma pharmacokinetics for multiple dose in healthy subjects:

Figure 5 shows plasma concentration vs. time curve for 7 day multiple dosing. The PK parameter for subjects receiving oral single dose of FIN for 7 consecutive days is described in Figure 6.

Plasma pharmacokinetics for multiple dose in healthy subjects:

Figure 5 shows plasma concentration vs. time curve for 7 day multiple dosing. The PK parameter for subjects receiving oral single dose of FIN for 7 consecutive days is described in Figure 6.

Plasma pharmacokinetics for multiple dose in healthy subjects:

Figure 5 shows plasma concentration vs. time curve for 7 day multiple dosing. The PK parameter for subjects receiving oral single dose of FIN for 7 consecutive days is described in Figure 6.

Plasma pharmacokinetics for multiple dose in healthy subjects:

Figure 5 shows plasma concentration vs. time curve for 7 day multiple dosing. The PK parameter for subjects receiving oral single dose of FIN for 7 consecutive days is described in Figure 6.

Plasma pharmacokinetics for multiple dose in healthy subjects:

Figure 5 shows plasma concentration vs. time curve for 7 day multiple dosing. The PK parameter for subjects receiving oral single dose of FIN for 7 consecutive days is described in Figure 6.

Plasma pharmacokinetics for multiple dose in healthy subjects:

Figure 5 shows plasma concentration vs. time curve for 7 day multiple dosing. The PK parameter for subjects receiving oral single dose of FIN for 7 consecutive days is described in Figure 6.

Plasma pharmacokinetics for multiple dose in healthy subjects:

Figure 5 shows plasma concentration vs. time curve for 7 day multiple dosing. The PK parameter for subjects receiving oral single dose of FIN for 7 consecutive days is described in Figure 6.

Plasma pharmacokinetics for multiple dose in healthy subjects:

Figure 5 shows plasma concentration vs. time curve for 7 day multiple dosing. The PK parameter for subjects receiving oral single dose of FIN for 7 consecutive days is described in Figure 6.

Plasma pharmacokinetics for multiple dose in healthy subjects:

Figure 5 shows plasma concentration vs. time curve for 7 day multiple dosing. The PK parameter for subjects receiving oral single dose of FIN for 7 consecutive days is described in Figure 6.

Plasma pharmacokinetics for multiple dose in healthy subjects:

Figure 5 shows plasma concentration vs. time curve for 7 day multiple dosing. The PK parameter for subjects receiving oral single dose of FIN for 7 consecutive days is described in Figure 6.

Plasma pharmacokinetics for multiple dose in healthy subjects:

Figure 5 shows plasma concentration vs. time curve for 7 day multiple dosing. The PK parameter for subjects receiving oral single dose of FIN for 7 consecutive days is described in Figure 6.

Plasma pharmacokinetics for multiple dose in healthy subjects:

Figure 5 shows plasma concentration vs. time curve for 7 day multiple dosing. The PK parameter for subjects receiving oral single dose of FIN for 7 consecutive days is described in Figure 6.
Finafloxacin (FIN, Figure 1) is a broad spectrum fluorquinolone (FQ) that belongs to a new 8- cyanofluoroquinolone subclass [1]. FIN contains a novel 6-cyano motif which endows it with a unique chemical profile that differentiates it from other marketed FQs [2]. FIN exhibited significant antibacterial activity against a wide range of gram-negative and gram-positive bacteria in vitro and in vivo. In this study, we evaluated FIN for its potential to treat urinary tract infections (UTIs) and other infections associated with low pH environments such as UTIs. We assessed FIN's pharmacokinetics and bacterial activity in healthy volunteers.

Methods

Study design and subjects: Urine from six healthy volunteers [7] was collected before and following an oral dose of 800mg FIN according to the following time intervals: 1. 0–1, 2. 0–4, 3. 0–8, 4. 0–12, 5. 24 to 24 h, and 6. 24 h to 48 h. Urine from 0 to 4 h was then tested for antibacterial activity.

Urinary pharmacokinetics

FIN pharmacokinetics following an oral dose of 800mg are summarized in Table 3. FIN reached mean peak levels of 130-μg/mL in urine collected between 4–9 h. Urine samples collected between 4–9 h and 12–24 h were used for antibacterial susceptibility testing.

Urinary bacterial activity

Urinary parameters and pharmacokinetics following 800mg dose are summarized in Table 4. FIN exhibited superior antibacterial activity to CIP and LVX in synthetic urine medium against a panel of uropathogens.

Results and Discussion

Conclusions

FIN exhibits superior antibacterial activity to CIP and LVX in synthetic urine medium against a panel of uropathogens. FIN was well tolerated in six healthy volunteers receiving an oral dose of 800mg. FIN exhibited superior antibacterial activity in ex vivo urine against a range of UTI pathogens and warrants further investigation for this indication.

Literature