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

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Abstract

Background: FIN is a novel fluorquinolone (FQ) belonging to a new brood of bacterial FQs. FIN contains a novel cyclic base component which offers improved antibacterial activity at slightly acidic pH (pH 5.0 – 6.0), under which other marketed FQs exhibit significantly reduced activity.

Methods: The ability of FIN, CLX, FIN, CIP, MOX and ENX to induce DNA cleavage from human topo II, E. coli DNA gyrase and E. coli topoisomerase IV was evaluated using sensitivity plates, colony counting assay. The selectivity of FIN for the bacterial enzymes (DNA gyrase and topoisomerase IV) was measured using the 

Results and Discussion

Finafloxicin (FIN), ciprofloxacin (CIP), moxifloxacin (MXF) and enoxacin (ENX) and the topoisomerase poison VP16 were titrated against human Topoisomerase II, bacterial DNA gyrase and topoisomerase IV (Table 3, Figure 2).

Introduction

Finafloxicin (FIN) (Figure 1) is a broad spectrum fluorquinolone (FQ) with excellent activity against a variety of gram-negative and gram-positive bacteria. FIN contains a novel cyclic 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.

In addition, FIN displayed an excellent safety profile in a wide range of studies, making it a leading candidate for the treatment of infections caused by atypical pathogens. The selective toxicity of FIN and other FQs was investigated by measuring their comparative activities against the eukaryotic and bacterial DNA topoisomerases, thereby elucidating their potential therapeutic applications.

Figure 1. Finafloxicin hydrochloride.

Table 1. Comparative DNA cleavage for FQs with VP16. The ratio value (selectivity index) is a measure of how well a particular FQ targets eukaryotic topo II relative to VP16. For example, with MFX the plateau saturation levels could not be determined and CL50 could not be extrapolated.

<table>
<thead>
<tr>
<th>Comparator</th>
<th>CDL (µg/mL)</th>
<th>CL50 (µg/mL)</th>
<th>Selectivity index (Ratio to VP16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VP16</td>
<td>1</td>
<td>25</td>
<td>1</td>
</tr>
<tr>
<td>CLX</td>
<td>50</td>
<td>250</td>
<td>10 - 50</td>
</tr>
<tr>
<td>FIN</td>
<td>100 - 250</td>
<td>200</td>
<td>8 - 250</td>
</tr>
<tr>
<td>MOX</td>
<td>500</td>
<td>100 - 250</td>
<td>100 - 250</td>
</tr>
<tr>
<td>ENX</td>
<td>Undetectable</td>
<td>Unknown†</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Table 2. Comparative cleavage data for E. coli DNA gyrase.

<table>
<thead>
<tr>
<th>Comparator</th>
<th>CDL (µg/mL)</th>
<th>CL50 (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIN</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>CLX</td>
<td>1</td>
<td>52</td>
</tr>
<tr>
<td>MOX</td>
<td>1</td>
<td>200</td>
</tr>
<tr>
<td>ENX</td>
<td>10</td>
<td>200</td>
</tr>
<tr>
<td>UNK</td>
<td>50</td>
<td>500</td>
</tr>
</tbody>
</table>

Results and Discussion

Activity of FQs against the topoisomerase poison VP16 against human Topoisomerase II. The human topo II isos forms were 250 ng plasmid DNA substrate for 30 min at 37ºC. The reactions were terminated with SDS (1%) and digested with protease K (25 µg/mL) for 30 min at 90ºC and run on 1% agarose gel. Cleavage of the human enzyme was quantified as a function of released single stranded DNA.

Enochinolol (O) DNA gyrase and DNA Topoisomerase IV: Supercoiled pHOT1 was relaxed 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.

Endpoints for topoisomerase inhibition

The cleavage detection limit (CDL) was defined as the lowest concentration of drug, yielding detectable cleavage product. The CL50 value, defined as the concentration that induces 50% maximum cleavage, was used as an additional endpoint for bacterial enzymes.

Activity of FQs against the topoisomerase poison VP16 (Table 3, Figure 2). When compared on the basis of CDL, the test compounds exhibited equivalent activity against E. coli DNA gyrase, with the exception of ENX which exhibited a 10-fold lower activity than CLX, FIN, CIP and MOX.

On the basis of CL50, CLX was the most potent inhibitor of E. coli DNA gyrase, this was followed by FIN, then CIP, then FIN and ENX.

Activity of FQs against E. coli topoisomerase IV (Table 4, Figure 3). FIN, CLX and MOX exhibited comparatively greater activity than CIP and ENX against topo IV. FIN exhibited equivalent activity to CLX and MOX against topo IV on the basis of CDL (all 1µg/mL) and greater activity than CLX and MOX on the basis of CL50 (Table 3). CIP and ENX were less potent than the other test compounds on the basis of both CDL and CL50.

FIN can be classified as a group 4 fluoroquinolone together with MOX and CLX as indicated by its comparatively high activity against both bacterial enzymes (dual target activity).

Conclusions

• The test FQs exhibited a range of selectivity against the human enzyme compared with the test FQs.
• Conversely, FIN was one of the most potent inhibitors of E. coli DNA gyrase and topo IV, exhibiting a comparatively high level of activity against both bacterial enzymes.
• These data indicate that FIN is at least as potent as a panel of clinically used FQs against bacterial type II topoisomerases and predict a low potential for topoisomerase associated toxicity.

Literature