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

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Abstract

Background: FIN is a novel fluoroquinolone (FQ) belonging to a new 8-cyano subclass. FIN exhibits optimal efficacy at a slightly acidic pH (5.0 - 6.0) under which other FQ show decreased activity. Because of this property, FIN is intended for therapeutic use against bacterial infections associated with an acidic environment. The selectivity of FIN for eukaryotic and bacterial DNA topoisomerase II enzymes was evaluated using quantitative plasmid DNA cleavage assays *in vitro*.

Methods: The ability of FIN, clinafloxacin (CLX), ciprofloxacin (CIP), moxifloxacin (MXF) and enoxacin (ENX) to induce DNA cleavage from human topo IIα; *E. coli* DNA gyrase and topo IV was quantified and compared based on the cleavage detection limit (CDL), defined as the lowest concentration yielding detectible cleavage product compared with that of the known topo II poison, etoposide (VP16). The CL50 value, defined as the concentration that induces 50% maximum cleavage, was used as an additional endpoint for bacterial enzymes.

Results: The activity of FIN against the human enzyme was 250-fold lower than that of VP16 and places it well amongst the other FQ (in terms of fold lowered activity against the human enzyme) *viz*; CLX (10 - 50), CIP (100 - 250), MXF (500) and ENX (no CDL detectable).
FIN, CLX, CIP and MXF exhibited a CDL of 1ng/mL against bacterial DNA gyrase, ENX exhibited lower activity (10ng/mL). FIN, CLX and MXF displayed comparable activity against topo IV (1ng/mL), while CIP (10ng/mL) and ENX (50ng/mL) were less active. CL50 (ng/mL) against gyrase and topo IV respectivly show that FIN (25, 8) was more active against both bacterial targets than CLX (10, 52), CIP (120, 200), MXF (70, 200) and ENX (50, 500).

Conclusions: These data indicate that FIN is highly selective for bacterial type II topoisomerases. FIN exhibited superior activity to the comparator FQs in terms of potency against the individual bacterial enzymes and its relative eqipotency against these dual targets.

Introduction

Finafloxacin (FIN, Figure 1) is a 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 (pH 5.0 – 6.0), under which other marketed FQs exhibit significantly reduced activity [2].

In addition, FIN displayed an excellent safety profile in a wide range of predictive, *in vitro*, toxicity assays [3] and was well tolerated in healthy human volunteers [4].

FQs target DNA bound, bacterial type II topoisomerase enzymes, forming a stable complex and halting DNA replication, resulting in the release of double stranded DNA breaks. The selectivity index of FIN and other FQs was investigated by measuring their comparative activities against the human counterpart of these target enzymes, topo IIα.

The relative target activities of FIN and other test FQs against their bacterial targets, were determined *in vitro*, against bacterial DNA gyrase and topoisomerase IV.

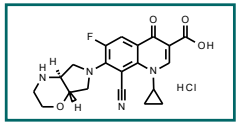

Figure 1. Finafloxacin hydrochloride.

Methods

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

Human Topoisomerase II: Human topo IIα isoform was added to 250 ng plasmid 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 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 detectible cleavage product.

The **CL_{50}** value, defined as the concentration that induces 50% maximum cleavage, was used as an additional endpoint for bacterial enzymes.

Results and Discussion

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

The human topo IIα isoform was sensitive to VP16 (CDL = 1 µg/mL, CL_{50} = 25 µg/mL). The activities (CDL and CL_{50}) of the test compounds were determined against the human enzyme for the calculation of their relative selectivity indices [activity of test FQ (CDL or CL_{50}) / activity of VP16 (CDL or CL_{50})].

Of these, CLX exhibited the lowest selectivity index (based on CL_{50}; 10, based on CDL; 50) against the eukaryotic enzyme. This was followed by CIP (8, 250) then FIN (100, 250) and MXF (500 - estimated). ENX exhibited the greatest selectivity index of the test compounds (both CDL and CL_{50} were undetectable). These data are summarized in Table 1 and Figure 2.

The CDL values of the FQs against human topo IIα indicate a range of selectivity indices spanning 50- 500-fold. FIN exhibited an index of 250 which placed it well 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

Figure 2. Electrophoretic separation of linear DNA fragments, released as a result of FIN (left) and CLX (right) inhibition of human topo IIα.

Comparator	CDL (µg/mL)	CL_{50} (µg/mL)	Selectivity index (Ratio to VP16)
VP16	1	25	1
CLX	50	250	10 - 50
CIP	100 - 250	200	8 - 250
FIN	250	500	100 - 250
MXF	500 - 1000	Unknown*	500 (estimated)
ENX	Undetectable	-	N/A

Table 1. Comparison of 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.
*In experiments with MXF the plateau saturation levels could not be determined and CL_{50} could not be extrapolated.

Comparator	CDL (ng/mL)	CL_{50} (ng/mL)
CLX	1	10
FIN	1	25
MXF	1	70
CIP	1	120
ENX	10	50

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

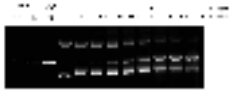

Figure 3. Electrophoretic separation of linear DNA released following FIN inhibition of DNA bound *E. coli* DNA gyrase.

Comparator	CDL (ng/mL)	CL_{50} (ng/mL)
FIN	1	8
CLX	1	52
MXF	1	200
CIP	10	200
ENX	50	500

Table 3. Comparative cleavage data for *E. coli* DNA topoisomerase IV.

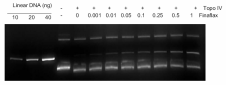

Figure 4. Electrophoretic separation of linear DNA released following FIN inhibition of DNA bound *E. coli* DNA topoisomerase IV.

When compared on the basis of CDL, the test compounds exhibited equipotent activity against *E. coli* DNA gyrase, with the exception of ENX which exhibited a 10-fold lower activity than CLX, FIN, CIP and MXF.

On the basis of CL_{50}, CLX was the most potent inhibitor of *E. coli* DNA gyrase, this was followed by FIN, then MXF, then CIP and ENX.

Activity of FQs against *E. coli* topoisomerase IV (Table 3, Figure 4).

FIN, CLX and MXF exhibited comparatively greater activity than CIP and ENX against topo IV. FIN exhibited equipotent activity to CLX and MXF against topo IV on the basis of CDL (all 1ng/mL) and greater activity than CLX and MXF on the basis of CL_{50} (Table 3). CIP and ENX were less potent than the other test compounds on the basis of both CDL and CL_{50}.

FIN can be classified as a group 4 fluoroquinolone together with MXF and CLX as indicated by its comparably high activity against both bacterial enzymes (dual target activity) [5].

Conclusions

- The test FQs exhibited a range of selectivity against human topo IIα compared with VP16.
- FIN exhibited a high selectivity index against the human enzyme compared with the test FQs.
- Conversely, FIN was one of the most potent inhibitors of *E. coli* 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

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