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

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Revised abstract

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* eradication. The antibacterial activity of FIN, was determined against FQ^{RES} and susceptible 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: MIC_{50} and MIC_{90} values of FIN and LVX for 31: (18 FQ^{RES} and 13 susceptible) strains are shown in Table 1. Additionally, MICs were determined for a panel of 24 FQ susceptible isolates (Fig. 2). Emergence of resistance was determined by pre-treating infected animals with sub therapeutic levels of FIN 1mg/kg or ciprofloxacin (CIP) 2.5mg/kg, (o.d., 7d) before treatment with FIN or CIP (10mg/kg, o.d., 7d). FIN cleared infection (negative urease test, 24h post-therapy) in 100% of pre-exposed animals whereas subsequent CIP treatment failed.

Conclusions: FIN exhibited increased efficacy at acidic pH compared to LVX. This was especially true against the FQ resistant strains. Additionally, FIN pre-exposure did not select for resistance *in vivo.* This unusual acid dependent activity seems particularly well suited for Helicobacter eradication and warrants a clinical evaluation.

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 (pH 5.0 – 6.0) under which other marketed FQs exhibit significantly reduced activity [2].

FIN also exhibited superior activity to comparator FQs against adherent bacteria *in vitro* [3] and in a wide range of rodent infection models [4,5]. Additionally, FIN displayed an excellent safety profile in a wide range of predictive, *in vitro,* toxicity assays [6] and was well tolerated in healthy human volunteers [7]. These attributes suggest that FIN warrants clinical investigation for bacterial infections that are associated with low pH such as urinary tract infection and *Helicobacter pylori* eradication.

FQs such a levofloxacin (LVX) have shown good antibacterial activity against *H. pylori* and a successful eradication rate when used in triple combination therapy. The antibacterial activity of FIN was investigated against FQ susceptible and resistant strains at acidic pH and against *H. felis* in a novel murine infection that was developed to be a stringent evaluator of anti-helicobacter therapy.

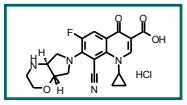

Figure 1. Finafloxacin hydrochloride.

Methods

Minimum inhibitory concentration (MIC) determination

MICs were determined for FIN and LVX against *H. pylori* strains (n= 55) that were obtained from patients gastroscoped in the Southwest of France. MICs were performed by agar dilution at 3 different pHs: 7.3, 6.3 and 5.3. An inoculum (equivalent to a McFarland 3 opacity standard) from a 48 h culture was plated on Mueller Hinton agar enriched with 10% sheep blood prepared extemporaneously and containing progressive concentrations of the FQs (0.015 - 128 mg/L). Reading was performed after 2 - 3 days of incubation at 37°C in a microaerobic atmosphere.

Murine model of *Helicobacter felis* infection

H. felis was passaged in female Swiss-Webster mice by repeated feeding of colonised gastric homogenate, achieving a persistent infection which could not be eradicated with conventional antibacterial monotherapy but could be eradicated following FIN monotherapy. Eradication was defined as a negative urease test on gastric tissue, 4 weeks post-therapy.

The propensity for resistance emergence to FIN and ciprofloxacin (CIP) was investigated by pre-treating infected animals (n = 5) with sub therapeutic doses of FIN (1mg/kg) or CIP (2.5mg/kg), (once daily, 7d) before treatment with therapeutic doses of FIN or CIP (10mg/kg, once daily, 7d). The therapeutic endpoint was defined by a negative urease test, 24h post treatment which could be attained, only if resistance to the test drug did not emerge in the colonising bacteria during pre-treatment.

Results and Discussion

Susceptibility of *H. pylori* isolates to FIN and LVX under standard conditions

In total, 55 *H. pylori* isolates were investigated for their susceptibility to FIN and LVX. Initially, a panel of 31 strains were investigated (Table 1). These were pre-defined as FQ susceptible (n = 18) or resistant (n = 13), based on their susceptibility to LVX.

Under standard susceptibility testing conditions (pH 7.3), FIN and LVX exhibited similar activities against the tested strains (Table 1).

Effect of pH on the activity of FIN against *H. pylori*

Agar dilution MICs were determined at pH 7.3, 6.3 and 5.3 against a second panel of 24 LVX susceptible strains. The antibacterial activity of FIN, as seen by its MIC distribution (Figure 2), increased in a step-wise manner as the pH became more acidic. Shifting from neutral to acidic pH had a minimal effect on the activity of LVX.

FIN exhibits improved antibacterial activity, under conditions of acidic pH, against a wide range of species [1,2]. Under the same conditions, other marketed FQs exhibit significantly reduced activity. This unusual property has been attributed, at least in part, to the relatively low intrinsic basic capacity (pK_a) of FIN compared to that of other FQs [1]. This most probably results in an increased cellular accumulation of FIN under acidic conditions. This is illustrated in Figure 3, in which a correlation is drawn between low intrinsic basic capacity of experimental and commercially available FQs and their improved therapeutic efficacy in an *in vivo* model of Helicobacter colonisation [8].

Results and Discussion

pH	Finafloxacin MIC_{50} [mg/L]	Finafloxacin MIC_{90} [mg/L]	Levofloxacin MIC_{50} [mg/L]	Levofloxacin MIC_{90} [mg/L]
FQ susceptible (n = 18)				
7.3	0.125	0.5	0.25	0.5
6.3	0.125	0.25	0.25	0.5
5.3	0.125	0.25	0.25	0.5
FQ resistant (n = 13)				
7.3	8	16	4	8
6.3	8	8	4	16
5.3	2	4	4	16

Table 1. MIC_{50} and MIC_{90} of finafloxacin (FIN) and levofloxacin (LVX) against a panel of fluoroquinolone susceptible (n = 18) and FQ resistant (n = 13) clinical isolates of *H. pylori.*

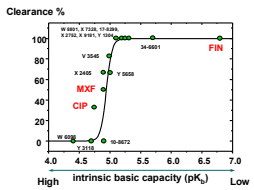

Figure 3. Capacity of various commercially available (CIP: ciprofloxacin, MXF: moxifloxacin) and experimental FQs (FIN: finafloxacin) to clear a *Helicobacter* infection in mice *vs.* the intrinsic basic capacity of the test compounds [8].

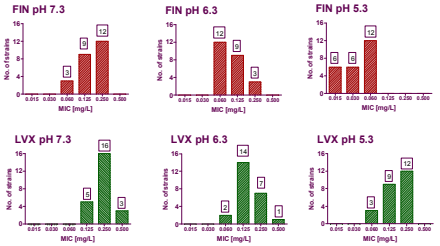

Figure 2. MIC distribution of FIN (top row) and LVX (bottom row) against 24 FQ susceptible *H. pylori* strains at pH 7.3 (left) pH 6.3 (middle) and pH 5.3 (right).

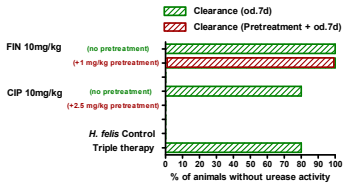

Figure 4. Clearance (as determined by negative urease test, 24h post-therapy) of persistent *H. felis* infection following seven day pre-treatment of mice with sub therapeutic doses of CIP (2.5 mg/kg, 7d) or FIN (1 mg/kg, 7d) before seven day treatment with therapeutic doses (10 mg/kg).

Murine model of *H. felis* infection

H. felis was passaged in mice to achieve a persistent infection that exhibited a similar response to therapy as *H. pylori* in humans. Triple therapy (bismuth citrate, amoxicillin (AMX) and metronidazole (14 d) could sucessfully eradicate infection (endpoint: negative urease test on gastric tissue, 4 weeks post-therapy) whereas monotherapies of clarithromycin, AMX or CIP all failed. FIN was the only drug able to successfully eradicate infection when administered as a monotherapy.

The present study was performed to investigate whether pre-exposure to FIN and CIP could select for resistance *in vivo* and lead to subsequent treatment failure. Both drugs could clear (negative urease test, 24 h post-therapy) infection from animals with no prior antibiotic exposure (Figure 4).

Sub therapeutic (FIN 1mg/kg or CIP 2.5mg/kg) doses were administered to infected mice, once daily for 7d. The mice were then administered therapeutic doses (10 mg/kg). The data for clearance in pre-exposed mice are summarised in Figure 4.

These findings show that pre-exposure to CIP leads to an total failure of the subsequent treatment (failure to clear infection in 100% of animals) where as pretreatment with FIN did not alter the success subsequent therapy (0% failure). Selection of resistance during pre-treatment was the most probable reason for the subsequent treatment failure seen with CIP.

Conclusions

- FIN exhibited improved antibacterial activity, *in vitro,* against a panel of both FQ resistant and susceptible recent clinical isolates of *H. pylori* at low pH.
- In addition to exhibiting clearly superior efficacy in an murine model of persistent Helicobacter infection, FIN (sub therapeutic dose) did not select for resistance in this model.
- The pH activation observed with FIN against *H. pylori in vitro* and its efficacy in a difficult to treat model of *H. felis* colonisation, suggest that FIN may be a promising treatment that could improve *H. pylori* eradication therapy in humans.

Literature

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