Finafloxacin HCl - a novel experimental fluoroquinolone with monotherapeutic potential for *Helicobacter pylori* eradication

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Abstract

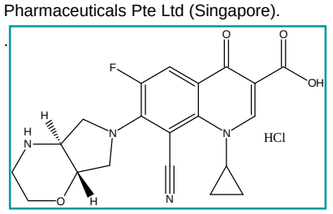
Finafloxacin HCl is a novel fluoroquinolone antibiotic under early stage clinical development by MerLion Pharmaceuticals as a potential monotherapy for the eradication of *Helicobacter pylori (H. pylori)*. Finafloxacin HCl has been shown to be equipotent to a range of marketed fluoroquinolones at pH 7.4, *in vitro*. However, at pH 5 it demonstrates markedly increased activity when compared with these agents.

The *in vitro* activity of finafloxacin HCl against *H. pylori* is similar to amoxicillin, clarithromycin and tetracycline - the most effective antibiotics used in combination for the eradication of *H. pylori* in man. Importantly, when head-to-head comparisons of finafloxacin HCl and these antibiotics were studied *in vivo* using a modified *Helicobacter felis* mouse model, finafloxacin HCl was unique amongst the agents in being able to eliminate the organism when dosed as a monotherapy. Finafloxacin HCL is more potent *in vitro* than metronidazole and is also effective against metronidazole-resistant and clarithromycin-resistant *Helicobacter* strains.

Although most antimicrobials are effective against *H. pylori in vitro, in vivo* activity is limited by a range of factors including the low pH environment of the gastric mucosa that reduces the effectiveness of these drugs. Finafloxacin HCl has a distinct pH/activity profile which may be used to overcome this barrier to efficacy.

Introduction

Finafloxacin HCl ($C_{20}H_{20}FClN_4O_4$, molecular weight 434.86) is a novel fluoroquinolone being developed as a treatment for the eradication of *H. pylori* by MerLion Pharmaceuticals Pte Ltd (Singapore).



Marketed fluoroquinolones show mildly basic pH optima, but lose their antibacterial activity under acidic conditions. Finafloxacin HCl has been shown to be equipotent *in vitro* to a range of marketed fluoroquinolones at 'physiological' pH, but shows markedly increased activity under lower pH conditions.

The improved MICs of finafloxacin HCl at low pH is important because the sites of many infections, such as urine and gastric mucosa, are characterised by an acidic pH.

Regimens currently used to eradicate *H. pylori* involve 'triple' or 'quadruple' therapy. Low rates of compliance due to a combination of complicated regimens, poor side effect profiles and cross-resistance highlight that this remains an area of unmet medical need.

Clinical treatment regimens are only relevant if an eradication rate of at least 80% can be achieved without major side effects and induction of pronounced bacterial resistance. (1)

None of the antibiotics currently approved for the treatment of *H. pylori* provide acceptable levels of eradication when used as a monotherapy. Finafloxacin HCl shows *in vivo* potential as such a monotherapy.

Based on 'best in class' pre-clinical efficacy and safety data, human clinical trials of this drug are underway.

Methods

MIC (*H. pylori)* : These were established using 5 x 10^5 bacteria/ml inoculum in brain-heart-infusion medium supplemented with 5% foetal calf serum (Boehringer, Manheim). Microtitre plates were incubated at 37ºc for 48 – 72hrs in an atmosphere containing 10% CO_2.

Helicobacter felis mouse model: Female Swiss-Webster mice (ca. 20 g body weight) were used. Donor mice were challenged once by gavage with 0.1 ml of a homogenate of infected stomach tissue (1 infected stomach homogenized in 10 ml BHI broth, 10^5-10^6 CFUs/ml) and sacrificed after 1-3 months. Gastric homogenate from donor mice was examined microscopically for the presence of highly motile *H. felis* cells and assayed for urease activity to estimate the infectious load. Groups of animals were then infected by a single challenge with gastric homogenate obtained from the donor mice. Therapies were initiated 3-5 days after challenge. Negative urease test results from stomach plugs at 4 weeks were used as a criteria for eradication.

Therapies used in *H. felis* model: Finafloxacin: *p.o.,* twice daily (*b.i.d)* for 10 days. Triple therapy: 6.2mg/kg bismuth citrate, 22.5mg/kg of metronidazole, 50mg/kg of amoxicillin trihydrate once daily (*q.d.*) for 10 days.

MIC (*E. coli, K. pneumonia, P. aeruginosa, S . aureus, P. vulgaris, P. mirabilis)*: Standard micro dilution methods according to CLSI guidelines were used. The pH of the growth medium (MH broth) was adjusted by the addition of HCl or NaOH.

Results and Discussion

Activity against *H. pylori*: The potency of growth inhibition by finafloxacin HCl was within the range demonstrated by the most effective antibiotics used for the eradication of *H. pylori* in man, and ciprofloxacin (Table 1). Finafloxacin HCl was also effective against a metronidazole-resistant strain and against at least 9 out of 10 clarithromycin-resistant strains.

Table 1: Finafloxacin HCl *in vitro* activity vs. *H. pylori* (n = 46)

Compound	MIC (µg/ml)		
	MIC50	MIC90	Range
Finafloxacin HCl	0.098	0.195	=0.049 - 6.25
Ciprofloxacin	0.195	0.39	=0.049 - 12.5
Azlocillin	0.098	0.195	=0.049 - 0.78
Metronidazole	1.56	3.12	0.195 - >10
Clarithromycin	=0.049	=0.049	=0.049 - 6.25

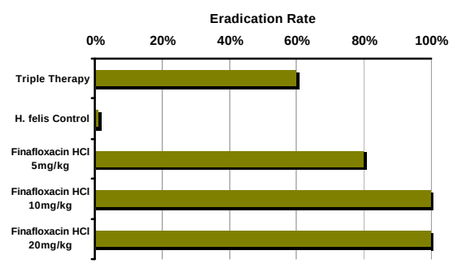
***In vivo* Activity:** Infection models in experimental animals bridge the gap between *in vitro* testing and the clinical evaluation of an antibiotic. However, because *H. pylori*, exhibits a limited host range, a *H. felis* model in mice was used to mirror the clinical ætiology of *H. pylori* in man (2). The model was adapted to mimic the pattern seen in humans where antibiotic monotherapy is ineffective but triple therapy gives eradication rates of approximately 80% (3).

Mice were infected using serially passaged *H. felis* taken directly from the stomach of previously infected mice rather than from culture. With each passage *H. felis* becomes better adapted to the mouse stomach and more refractory to treatment until only triple therapy approaches are effective. If passaged further, *H. felis* cannot be eradicated even with triple therapy.

In this model, triple therapy gave an eradication rate of 60% slightly below than that seen in a clinical setting. Finafloxacin HCl at 10 and 20mg/kg *b.i.d*. for 10 days showed 100% eradication of *H. felis* (Figure 1).

Finafloxacin HCl was also more effective than monotherapy with amoxicillin, azlocillin, ciprofloxacin, metronidazole and clarithromycin (results not shown). These comparator antibiotics showed no eradication when used as a monotherapy. Experiments with further passaged *H. felis* showed eradication rates of 100% using 12.5mg b.i.d. finafloxacin HCl for 14 days compared with only 30% using 14 days of triple therapy *q.d.* (results not shown).

Figure 1: *In vivo* evaluation of finafloxacin HCl *b.i.d* 10 days *versus H. felis*



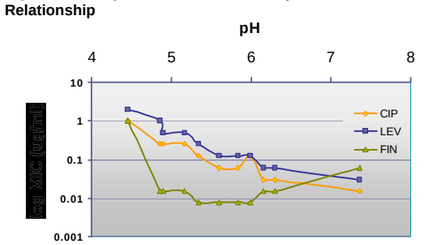
Effect of pH on Activity: Fluoroquinolones may only diffuse through bacterial membranes to reach their molecular targets when in an uncharged state. The uncharged state of finafloxacin HCl is stabilised under pH conditions below neutral. Therefore, "standard" MIC values, generated at near-neutral pH range tend to underestimate the relative efficacy of finafloxacin HCl under *in vivo* conditions within the acidic gastric mucosal niche of *H. pylori.*

Table 2: Relationship of MIC and pH (4)

Organism	Strain	Finafloxacin HCl Minimum MIC (µg/ml)	pH Range of Min MIC	MIC Cipro / MIC Fina	MIC Levo / MIC Fina
E. coli	ATCC 25922	0.0078	5.3 - 6	8 - 16	16 - 32
E. coli	ATCC 700928	0.015	5.8 - 6.2	4 - 16	8 - 16
E. coli	ATCC 10536	0.0078	5.6 - 6.2	2 - 4	4 - 16
K. pneumonia	BAT 39	0.03	5.6 - 6.2	4 - 8	8 - 16
P. aeruginosa	ATCC 27853	1	4.9 - 6	2 - 8	2 - 8
P. aeruginosa	PA01	0.5	4.9 - 6.2	0.25 - 1	1 - 4
S. aureus	ATCC 29213	0.03	6.2	16	16
S. aureus (MRSA)	ATCC 33591	0.06	5.6 - 6.3	4 - 8	4 - 8
P. vulgaris	ATCC 33420	0.125	4.9 - 6	1 - 8	1 - 16
P. vulgaris	DSM 2140	0.125	4.9	8	16
P. mirabillis	DSM 788	0.25	4.9 - 6	0.5 - 4	1 - 4

The pH/ activity profile of finafloxacin HCl is distinct from that of all fluoroquinolones tested. This effect was confirmed across a range of bacteria (Table 2). Figure 2 shows an example of the pH activity relationship.

Figure 2: Example: *E. coli* ATCC 25922 pH / MIC Relationship



Conclusions

- Finafloxacin HCl shows a pH dependent activity which is the opposite of marketed fluoroquinolones. Its activity is enhanced at lower pH.
- Finafloxacin HCl has shown equivalent *in vitro* activity to the commonly used antibiotics in triple therapy and shows a low cross resistance with clarithromycin.
- Finafloxacin HCl shows higher eradication rates in an adapted *H. felis* model than triple therapy.
- Overall, finafloxacin HCl appears to be a promising potential monotherapy for *H. pylori* eradication.

References

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