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

R. Endermann[1], C. Ladel[1], W. Stubbings[2], H. Labischinski[2];

[1]Bayer Health Care AG, Elberfeld, Germany, [2]MerLion Pharmaceuticals Pte Ltd, Singapore, Singapore.

Contact information:

RAINER ENDERMANN
Bayer HealthCare AG,
Elberfeld, 0403
Germany

rainer.endermann@bayerhealthcare.com

Abstract

Background: Finafloxacin (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 (LVX) and moxifloxacin (MXF), in a wide range of *in vivo* models.

Methods: Female CFW-1 mice (n = 6) were used. Bacterial inocula were administered by the following routes: intraperitoneal, oral application, implantation of colonised catheter material or direct injection into the kidney, bladder, thigh, granuloma pouch or abscess. Treatment was commenced 0.5 - 3h postinfection. End points were determined by % survival (at 3 - 5 days) or by reduction of bacterial counts (Δ $\log_{10}$ CFU/mL) in homogenised tissue.

Results: FIN (10mg/kg s.c.) exhibited greater killing of *S. aureus* in the thigh muscle (Δ $\log_{10}$ CFU/mL: FIN > -4, MXF -3, CIP -1 and LVX -2) than the comparators. FIN (10mg/kg p.o.) exhibited equal killing of *S. aureus* (Δ $\log_{10}$ CFU/mL: FIN -2, MXF -2) and greater killing of *P. aeruginosa* (Δ $\log_{10}$ CFU/mL: FIN -1, CIP, LVX and MXF all <(-1)) than the other FQs in infected abscess models.
FIN (10mg/kg p.o.) exhibited bactericidal activity in severe *E. coli* pyelonephritis (Δ $\log_{10}$ CFU/mL; FIN -4, CIP -3, LVX -4, MXF -3) and also in ascending *P. mirabilis* cystitis (Δ $\log_{10}$ CFU/mL: FIN and CIP (100mg/kg p.o.) -4). Data not shown.
Additionally, FIN exhibited equal, if not superior activity to the comparators in granuloma pouch and implanted catheter models (*S. aureus*), enteritis (*S. typhimurium*), and post surgical polymicrobial peritonitis.

Conclusion: The superior efficacy of FIN over CIP, LVX and MXF in these difficult to treat models was in line with their respective MICs at lower pH values which are anticipated to occur in many infection models, especially those involving inflammation (abscess) or low pH fluids such as urine. These data suggest that the pH activity profile of FIN may be an advantage in combating severe bacterial infection.

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 excellent activity against adherent bacteria *in vitro* [3].

Additionally, FIN displayed an excellent safety profile in a wide range of predictive, *in vitro,* toxicity assays [4] and was well tolerated in healthy human volunteers [5]. 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.

FIN was a highly efficacious agent in eradicating Helicobacter spp. from a very difficult to treat murine model [6]. To further examine its therapeutic potential, FIN was evaluated in a number of rodent models of infection that were selected to offer a wide range of infection sites and to mimic the type of infections that are difficult to treat in humans, including those involving formation of adherent bacterial populations.

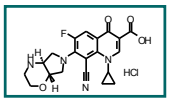


Figure 1. Finafloxacin hydrochloride.

Methods

Thigh Infection Model - *S. aureus.* Mice were rendered neutropenic by the subcutaneous (s.c.) administration of cyclophosphamide 4 days and 1 day prior to infection. Under light anaesthesia (CO_2) 0.1 mL of a log-phase culture of *S. aureus* DSM 11823 (3 x 10^7 CFU/mL) was injected into the right hind leg. Treatment started 30 min post infection (P.I.) with a second dose at 4 h P.I. Mice received doses of 2, 10 and 50 mg/kg s.c. 24 h after infection mice were killed and the number of bacteria remaining in the thigh homogenates was determined by plating out dilutions for CFUs.

Implanted Foreign Body Model - *S. aureus* Catheters were incubated overnight in a *S. aureus* DSM 11823 culture. The catheter was rinsed and then implanted s.c. in mice. Treatment started 3 h later and continued BID until the day prior to removal, with 10 mg/kg/dose. Samples were removed at 4 and 7 days. The explants were homogenised (Ultra Turrax), 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 s.c. on the back of mice. Within 3 days a capsule formed around the implant and this was infected with 1 x 10^5 *S. aureus* or 4 x 10^4 *P. aeruginosa*. Treatment was with 10 mg/kg 2 h post infection.

Postoperative polymicrobial sepsis. (Caecal ligation and puncture model) Mice were anaesthetised and the peritoneum opened with a small cut. The caecum was moved out of the peritoneum. The caecum was ligated and punctured with a 21G needle. The ligated intestine was replaced and the wound closed. 3 doses of 10 mg/kg were given at 4, 18 and 24 h post operation. Efficacy was determined by survival over 7 days.

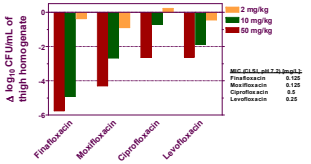
***E. coli* pyelonephritis.** Mice were anaesthetised, the right flank shaved to locate the kidney. Holding the kidney below the skin, 10 µL of a suspension of *E. coli* DSM 10650 (1 x 10^5) was injected directly into the kidney by using a 21G needle. Mice were treated with a single dose of 1 or 10 mg/kg 2 h P.I. 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 staphylococci in the thigh tissues is shown in Figure 2. FIN produced a dramatic fall of 5 $\log_{10}$ CFU recovered from the thigh homogenates at 10 mg/kg, far more than was seen with the other compounds. FIN and MXF had the lowest MICs (0.125 mg/L) but MXF, although being more active than CIP and LVX, was less active at all dose levels than FIN.

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

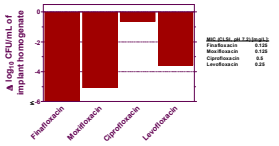


Results and Discussion

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). MXF was also effective but was still inferior to FIN. LVX had an intermediate effect.

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



Infected abscess model (Gelfoam™) – *S. aureus* and *P. aeruginosa.*

Figure 4 shows the reduction in the numbers of *P. aeruginosa* remaining at Day 7 P.I. FIN was the most effective of the four compounds and MXF the least active.

Organs were also sampled in the mice infected with *S. aureus* and Figure 5 shows the reduction in CFU in the abscess and the various organs. Only FIN and MXF were tested and both were effective in reducing CFU counts in the various tissues..

Figure 4. *P. aeruginosa* reduction in abscesses.

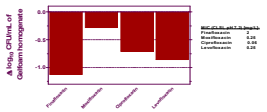
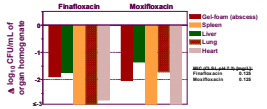


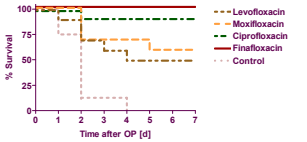
Figure 5. *S. aureus* reduction in various tissues.



Postoperative Polymicrobic Sepsis Model.

The survival of groups of 10 mice treated with three doses of 10 mg/kg of the four FQs is shown in Figure 6. By 7 days post operation all the mice treated with FIN were alive. CIP although being slightly less effective, protected 90% of mice. MXF and LVX were less protective

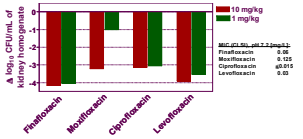
Figure 6. Postoperative Polymicrobic Sepsis Model – survival over 7 days post operation. (N=10).



***E. coli* pyelonephritis**.

FIN was the most active of the four FQs tested, with a 4-log CFU reduction in the kidneys at 2 days P.I. with 10 mg/kg. LVX was equally effective, CIP and MXF were less effective.

Figure 8. *E. coli* pyelonephritis.



Other models.

A variety of other models were used, including granuloma pouches in mice, ascending pyelonephritis using *P. mirabilis*, a peritoneal infection with *L. monocytogenes*, the eradication of *H. felis*, *Salmonella typhimurium* infection and an LPS induced shock model. With the exception of the *P. mirabilis* infection, FIN was the most active compound. The results of all the experiments are summarised in Table 1.

Table 1. Summary of *In vivo* test results

Infection model	Species	Pathogen	Efficacy ranking
UTI (Pyelonephritis)	Mouse	*E. coli*	**FIN** = LEV ≥ CIP > MOX
UTI (ascending)	Mouse	*P. mirabilis*	CIP > **FIN**
SSSI (Infected abscess)	Mouse	*S. aureus*	**FIN** > MOX
	Mouse	*P. aeruginosa*	**FIN** > LEV ≥ CIP > MOX
SSSI (Pouch)	Mouse	*S. aureus*	**FIN** ≥ MOX ≥ LEV
SSSI (Thigh muscle)	Neutropenic mouse	*S. aureus*	**FIN** >> MOX > LEV > CIP
SSSI (Foreign body)	Mouse	*S. aureus*	**FIN** = MOX ≥ LEV >> CIP
	Mouse	*P. aeruginosa*	**FIN** = LEV = CIP >> MOX
Peritonitis	Mouse	*L. monocytogenes*	**FIN** > MOX >> LEV = CIP
Salmonellosis	Mouse	*S. typhimurium*	**FIN** = MOX = CIP > LEV
Helicobacter eradication	Mouse	*H. felis*	**FIN** >> CIP = MOX
Polymicrobial sepsis	Mouse		**FIN** ≥ CIP > MOX > LEV
LPS - induced shock	Mouse	LPS	**FIN** ≥ MOX = CIP

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 MXF.
- The efficacy of FIN was better than expected from its MIC at pH 7.2, this 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 *ex vivo* toxicity tests, indicate that FIN is an excellent candidate for progression to the clinic.

Literature

[1] Wohlert *et al.*, 48th ICAAC, Washington DC 2008, Poster No. F1-2036.
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[3] Goh *et al.*, 48th ICAAC, Washington DC 2008, Poster No. F1-2042.
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[5] Patel *et al.*, 48th ICAAC, Washington DC 2008, Poster No. F1-2048.
[6] Buissonière *et al.*, 48th ICAAC, Washington DC 2008, Poster No. F1-2038.