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Efficacy of the Investigational Fluoroquinolone Finafloxacin in a Murine Inhalational Model of Melioidosis

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Abstract

Background: Finafloxacin (FIN) is a novel fluoroquinolone (FQ) in clinical development. It has the unique property of enhanced activity under acidic conditions, unlike other marketed FQs. *Burkholderia pseudomallei*, the causative agent of the disease melioidosis, is an intracellular pathogen that can replicate within host organelles where the local pH is acidic. Other FQs have reduced antibacterial activity at lower pHs and therefore FIN may offer greater efficacy in the treatment of intracellular infections, especially since it has been shown that FIN is efficiently accumulated within cells in an acidic environment. This study was performed to determine the efficacy of FIN in a murine inhalational model of melioidosis.

Methods: The efficacy of FIN against *B. pseudomallei* K96243 was investigated. This efficacy was determined *in vitro*, using time kill assays at 4 times the MIC, and *in vivo* against an inhalational challenge of *B. pseudomallei*. Mice were challenged with an average retained dose of 3.76 x 10^2 CFU and treated 6 hours post challenge with 50 mg/kg FIN, or ciprofloxacin and 240 mg/kg co-trimoxazole. Mice were treated twice a day for 14 days. Groups of mice were culled at 24 hours post-challenge and at cessation of therapy, and livers, lungs and spleens harvested to determine bacterial load. Other groups of animals were monitored for 8 weeks to determine protective efficacy.

Results: FIN demonstrated bactericidal activity against *B. pseudomallei* at both pH5 and pH7, this activity greatest at pH5 with complete kill at 24 hours. It also demonstrated a high level of protection against an inhalational challenge with *B. pseudomallei*. Treatment with FIN reduced the bacterial load in organs of infected animals culled 24 hours post-challenge. Bacterial burden was significantly reduced compared to ciprofloxacin or co-trimoxazole treated animals (P<0.05). Following 14 days of therapy 3 out of 5 animals dosed with FIN had cleared the infection from the organs harvested.

Conclusions: FIN has good antimicrobial activity against *B. pseudomallei*, determined *in vitro* and *in vivo*. Additional studies are warranted to optimise the dosing regimen.

Introduction

• Finafloxacin (FIN, Figure 1) is a novel, broad spectrum fluoroquinolone (FQ) [1] which is currently undergoing phase II and phase III clinical assessment in different indications.

• FIN exhibits the unusual property of enhanced *in vitro* and *in vivo* activity at slightly acidic conditions (pH 5.0 – 6.0) under which other marketed FQs show significantly reduced activity [2]. This is also true for adherent, slowly growing and intracellular bacteria [3,4].

• FIN displayed an excellent safety profile in a wide range of predictive, *in vitro*, toxicity assays, *in vivo* animal models and in humans [5].

• FIN may be advantageous against bacterial species, like *Burkholderia pseudomallei* and *Burkholderia mallei*, that are able to invade and spread intracellularly within the acidic environment that exists in the organelles of a host, for example, within phagosomes. Diseases caused by these organisms are notoriously difficult to treat. Here, we present preliminary *in vitro* and *in vivo* data suggesting a potential use of FIN in the treatment of such infections.

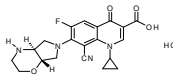


Figure 1
Finafloxacin hydrochloride

Methods

The study was an inpatient, randomized, double-blind, placebo-controlled, dose-escalating study to evaluate the safety, tolerability and pharmacokinetic profiles of single and multiple doses of FIN administered intravenously to healthy male and female subjects, aged between 18 and 55 years. Subjects received single doses of 200, 400, 600, 800 or 1000 mg and for the multiple dose study, subjects received 7 daily doses of 600, 800 or 1000 mg.

Blood and urine samples were collected prior to the study, at entry and at the end of evaluation for clinical chemistry, haematology and urinalysis. Plasma and urine samples were collected at various intervals from pre-dose until 48 h after initiation of dosing for pharmacokinetic analysis.

FIN concentrations were estimated in plasma and urine samples by a validated LC/MS-MS method. The lower quantification limit was 5 ng/mL in plasma and 100 ng/mL in urine. Pharmacokinetic parameters were evaluated using non-compartmental analysis. PK parameters, urinary recovery and renal clearance were determined.

All adverse events were coded according to MedDRA, reported and assessed as mild, moderate or severe. Test results of clinical laboratory, vital sign measurements, and ECG rhythm measurements (at screening, pre-dose, at various time points post-start infusion and 5-7 days post-dosing) were listed as actual results and changes from the baseline.

Results

Clinical laboratory evaluations:

Isolated, minor deviations from the normal ranges were observed for various haematology, blood chemistry and / or urinalysis parameters at various time points during the study. None of these deviations were considered clinically significant and no treatment or dose related trends were detected.

Plasma pharmacokinetics (PK) for single dose:

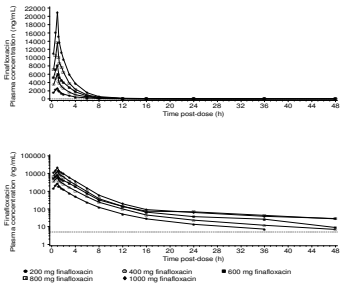
The PK values for subjects receiving single doses are tabulated in Figure 2. Plasma concentrations *vs.* time profiles of escalating single doses are shown in Figure 3.

Figure 2 Non-compartmental PK parameters determined as mean ± standard deviation in the subjects receiving single doses of FIN intravenously.

Dose	200 mg	400 mg	600 mg	800 mg	1000 mg
C_{max} [µg/mL]	2.6 ± 0.3	6.1 ± 10.4	8.1 ± 1.4	13.7 ± 23.6	21.0 ± 6.3
$AUC_{0\text{-}24}$ [h*µg/mL]	6.6 ± 0.7	14.3 ± 1.9	21.2 ± 2.3	31.2 ± 11.8	47.1 ± 16.3
$t_{1/2}$ [h]	12.1 ± 6.0	12.5 ± 3.7	10.3 ± 1.4	19.9 ± 9.8	20.6 ± 13.5

Results

Figure 3 Plasma concentration over time for single dose of FIN



C_{max} and $AUC_{0\text{-}24}$ increased almost linearly with dose. The median value of total body clearance was 27.2 and 24.9 L/hr for 400 mg and 800 mg dose, respectively.

Figure 4 Plasma concentration over time for multiple doses of FIN

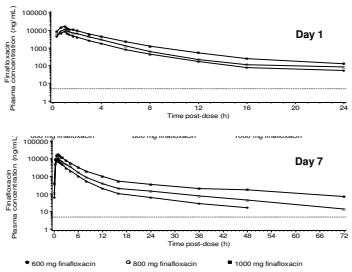


Figure 5. Non-compartmental PK parameters determined as mean ± standard deviation in the subjects on day 7 receiving multiple doses of FIN.

Dose	600 mg	800 mg	1000 mg
C_{max} [µg/L]	9.3 ± 1.7	11.9 ± 2.7	18.9 ± 6.3
$AUC_{0\text{-}24}$ [h*µg/L]	25.0 ± 6.7	37.1 ± 13.1	67.8 ± 22.6
$t_{1/2}$ [h]	22.1 ± 7.7	23.6 ± 4.6	32.4 ± 25.6

Steady state was reached at day 4, 72 h after first infusion. The median value of total oral body clearance was 24.6 and 15.6 L/hr for 600 mg and 1000 mg dose, respectively.

The majority of FIN renally eliminated as unchanged drug was excreted within 0 to 8 hours post-start of infusion. The mean fraction of the dose excreted in the urine ranged from 29.9% to 44.2% for single doses and 31.8 – 35% for multiple doses on day 7 of FIN.

Safety & Tolerability:

- Single (200 to 1000 mg) and multiple IV doses (600 to 1000 mg o.d. for 7 days) of FIN were considered to be safe and well tolerated by healthy volunteers.
- Overall, 85 treatment emergent adverse events (TEAEs) were reported in the study. 52 (37 for FIN and 15 for placebo) of those were suspected to be study-drug related (Figure 7). No dose-related trends were observed and a similar distribution of TEAEs was recorded for the different FIN dose groups or placebo (Figure 6). All adverse events with the exception of one were resolved without treatment.
- No deaths or serious adverse events were reported. One adverse event in the placebo dose group led to discontinuation.
- No treatment or dose-related trends in the 12-lead ECG parameters were noted. In particular, there was no evidence of prolongation of QTc interval at each dose level of FIN. There were no clinically important findings in the morphology of the 12 lead ECG for individual subjects at each dose level of FIN.

Figure 6. Summary of Treatment Emergent Adverse Events

Dose (mg)	Placebo	200	400	600	800	1000
Part A	3 (20%) [4]	1 (17%) [1]	2 (33%) [2]	0 (0%) [0]	2 (33%) [2]	2 (33%) [2]
Part B	5 (63%) [18]	-	-	9 (56%) [17]	6 (75%) [20]	6 (75%) [19]

Number of Subjects (%) with Adverse Events; [Number of Adverse Events]

Figure 7. Study drug related adverse events reported for subjects receiving single and multiple IV doses of FIN and placebo.

Body system	Part A FIN n = 18	Part A Placebo n = 15	Part B FIN n = 32	Part B Placebo n = 8
CNS	0 (0%) [0]	0 (0%) [0]	9 (28%) [12]	3 (38%) [3]
GI	4 (22%) [4]	1 (7%) [1]	5 (16%) [8]	4 (50%) [5]
Musculoskeletal	0 (0%) [0]	0 (0%) [0]	2 (6%) [4]	2 (25%) [3]
General	0 (0%) [0]	0 (0%) [0]	4 (13%) [4]	0 (0%) [0]
Infections	0 (0%) [0]	0 (0%) [0]	1 (3%) [1]	0 (0%) [0]
Metabolism	0 (0%) [0]	0 (0%) [0]	1 (3%) [1]	0 (0%) [0]
Psychatric	0 (0%) [0]	0 (0%) [0]	0 (0%) [0]	1 (13%) [1]
Respiratory	0 (0%) [0]	0 (0%) [0]	1 (3%) [1]	0 (0%) [0]
Skin	1 (6%) [1]	0 (0%) [0]	0 (0%) [0]	1 (13%) [1]
Vascular	0 (0%) [0]	0 (0%) [0]	1 (3%) [1]	0 (0%) [0]
Renal	0 (0%) [0]	1 (7%) [1]	0 (0%) [0]	0 (0%) [0]

Part A = Single dose Part B = Multiple dose in healthy subjects
n = Number of subjects [Number of Adverse Events]
CNS = Central nervous system, GI = Gastrointestinal

Conclusions

- This FIN Phase I study in healthy subjects revealed a favorable pharmacokinetics profile with high values for C_{max} and $t_{1/2}$. Appr. 30-40% of FIN were renally eliminated as unchanged drug.
- FIN was well tolerated following single dose and when given for seven days at a range of doses up to 1000 mg. Human safety data do not suggest any quantitatively higher or qualitatively different toxicity for FIN as compared with placebo and no dose-related trends were observed.
- Overall, these findings indicate that the risk of serious adverse reactions to FIN can be expected to be very low. Given the possible therapeutic effects of FIN, further clinical development of the drug appears to be justified and can be recommended.

Literature

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[4] Lemaire *et al.*, Int J Antimicrob Agents. 38 (2011) 52-9
[5] Patel *et al.*, AAC 55 (2011) 4386-93