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In Vitro Toxicological Profiling of Finafloxacin

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Abstract

Background: 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 show decreased activity and is therefore intended, e.g., for treatment of *H. pylori* and UTI. Several novel FQs have recently failed during development or shortly after launch due to safety / toxicology concerns. Therefore, prior to start of formal GLP safety / tox studies, FIN was rigorously profiled for the most common class related side effects against a series of predictive in *vitro* tests alongside comparator FQs; Ciprofloxacin (CIP), Trovafloxacin (TRO), and Sparfloxacin (SPA) and standard test controls, where appropriate.

Methods: Cytotoxicity potential was determined against the mouse macrophage line J774.AI and phototoxicity potential against the mouse fibroblast Balb/c 3T3 following exposition to UV irradiation.

Neurotoxic effects like excitatory potentials were tested in extracellular recordings from slices of rat hippocampus and the affintity to the GABA-A receptor were tested in the guinea pig ileum. Chondrotoxicity potential was studied on primary cartilage cells from dog and man and hepatotoxicity potential on primary rat hepatocytes. Cardiotoxicity (incl. QT effects) was investigated in Langendorff heart preparations and hERG channel experiments.

Results and Conclusions: FIN was examined in a series of *in vitro* cell or tissue based assays that are believed to be predictive of the most common, FQ-associated undesirable side effects. Under the conditions of these assays, FIN displayed a profile indicative of a low potential for the toxicity issues often associated with FQs.

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 exhibits superior activity compared with comparator FQs against adherent bacteria *in vitro* [3] and in a wide range of rodent infection models [4,5]. These attributes suggest that FIN warrants clinical investigation for bacterial infections that are usually associated with low pH such as urinary tract infection and *Helicobacter pylori* eradication.

Several novel FQs have failed at late stages of development or shortly after release due to concerns over safety / toxicology. Therefore toxicological profiling of FIN was addressed during early stages of development in an extensive set of predictive, *in vitro* toxicity assays.

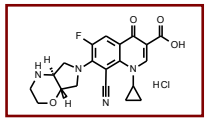

Figure 1. Finafloxacin hydrochloride

Methods

Mouse cytotoxicity – A permanent mouse macrophage cell line (J774.A1) obtained from ATCC was incubated with compounds for 72 h in DNEM in microtitre trays. After washing, cell viability was determined using a neutral red assay.

Excitatory and neurotoxic potential – Brains were removed from young rats and cooled immediately. Slices (450 µm) were made from the hippocampus and incubated in carbon saturated artificial cerebral spinal fluid (ACF) at RT. They were used within 1-2 h. A superfusion chamber was used at 34°C controlled at 2 mL/min ACF. A conventional electrophysiological method was used, with extracellular recordings being made from the pyramidal cell layer. Electric stimulation of 30 min was used and control conditions recorded for 30 min. Slices were then perfused with 2 µM of the test compounds and observed for a further 30 min.

Phototoxicity was determined in a permanent mouse fibroblast cell line (3T3 cells) obtained from ATCC and incubated in DMEM in microtitre trays. Compounds were added for 1 h and the trays exposed to UV irradiation for 20 min or 60 min.

Hepatotoxic potential – Liver perfusion was carried out in Wistar rats and the freshly isolated primary hepatocytes, washed in HBSS and stained with trypan blue to estimate viability (c 80%). A collagen sandwich gel using 8-well plates was used. Cells were incubated for up to 7 d with 7 d recovery. Cytotoxicity was determined by measuring aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH). Mitochondrial dehydrogenase activity was also measured using the MTT assay.

Chondrotoxic potential – primary cartilage cells from dogs and man were used. Cartilage from the knee joints of a 9 month old beagle dog. Human cartilage tissue was from a 62 year old female undergoing orthopaedic surgery. Direct cytotoxicity estimated by viability (neutral red technique, measured quantitatively with a spectrometer) of cartilage cells. More detailed studies included measurement of mitochondrial dehydrogenase activity (incubated with MTT for 2 h, measured quantitatively with a spectrometer), mitochondrial status (incubation with tetramethylrhodamine for 30 mins, measured with a fluorospectrometer), intracellular ATP (chemiluminescence assay, ATPLite assay), cell proliferation (^{14}C thymidine uptake measured at 2, 24, 48 and 72 h and scintillation counter used to determine uptake) and collagen type II content (cell-ELISA assay over 72 h using monoclonal antibodies and specific epitopes for collagen II).

Affinity to the GABA-A receptors from guinea pig ileum - Portions (4 cm long) from the terminal ileum of freshly killed guinea pigs were used, placed in an organ bath and contractions measured by multichannel recorders. The response to muscimol (EC_{50}) in the presence and absence of FIN at a range of concentrations up to 10^{-4} M was determined.

Cardiotoxic effects - Langendorff heart preparation. Hearts were removed from 3 male guinea pigs (370-410 g, Charles River) and perfused continuously for 15 min with drugs at 10^{-4} and $3x10^{-4}$ M. Left ventricular pressure, heart rate and coronary flow was measured.

hERG channel activity hERG currents were recoded from stably transfected HEK293 cells. FIN was compared with SPAR. Cells were transferred to a recording chamber and continuously perfused with 1-2 mL/min bath solution at RT. A whole cell patch-clamp configuration was established and recordings made. Concentrations used were FIN 300 µM. Sparfloxacin (SPA) 1, 10, 30, 100 and 300 µM. Changes in the magnitude of the current was measured.

Results and Discussion

Mouse cytotoxicity.

FIN had a very low cytotoxic effect, with an EC_{50} of 100 µg/mL and a NOEC of 30 µg/mL.

Phototoxicity – Mouse Fibroblasts.

FIN was classified as non-phototoxic in this system with an EC_{50} of >100 µg/mL after both 20 min and 1 h exposure. CIP was classified as slightly phototoxic (EC_{50} of >50 µg/mL).

hERG channel activity.

hERG channel is responsible for the potassium influx during repolarisation and an inhibition induces effects like QT-prolongation; this was not influenced by FIN up to 300 µM for outward and tail current. SPA had a concentration dependent effect; outward currents were blocked at an IC_{50} of 21.5 µM and tail currents at 25.4 µM.

Primary rat hepatocytes.

FIN was not hepatotoxic at concentrations of up to 100 µg/mL in all 4 test systems. In contrast trovafloxacin (TVO) produced an increase in ALT (Figure 2), LDH, and the MTT test. These results indicate that FIN has a low potential for hepatotoxicity.

Figure 2. Effect of FIN (left) and TVO (right) on rat hepatocyte ALT levels.

Primary human and dog chondrocytes. FIN showed no toxicity in any of the tests used, resulting in a NOEC level of >100 µg/mL. These are summarised in Figure 3. In contrast, CIP had a NOEC of 10 (dog) and 30 µg/mL (human).

Assay	Dog Chondrocytes	Human Chondrocytes
Neutral red	103 ± 6	94 ± 2
MTT Assay	105 ± 2	95 ± 5
Rhodamine assay	88 ± 5	102 ± 2
ATP	101 ± 12	100 ± 20
Proliferation assay	95 ± 9	97 ± 2
Collagen II determination	120 ± 6	106 ± 0.5

Figure 3. Effect of FIN (100 µg/mL) on Dog and Human Chondrocytes. Expressed at % of control cells.

Langendorff guinea pig heart preparation

No significant change in the left ventricular pressure, its rate of maximal pressure rise, heart rate and coronary flow was noted (p>0.05). No signs of cardiac arrhythmia were observed, NOEC = >300 µM. (Figure 4).

	% change in cardiac parameters				
M (Conc.)	Left Ventricular Pressure	dP/dtmax rate of max. pressure rise	Heart Rate	Coronary Flow
10^{-4}	-4 ± 2	0 ± 0	-3 ± 2	-2 ± 3
3×10^{-4}	+2 ± 3	+5 ± 3	-1 ± 1	+11 ± 9

Figure 4. Effect of FIN on Langendorff heart preparation.

GABA-A receptors affinity – guinea pig ileum.

These receptors are in the post-ganglionic cholinergic nerve endings in the ileum. When stimulated by the GABA-A receptor agonist, muscimol, contractions occur mediated by acetylcholine released by neurones. FIN had no effect on the EC_{50} of muscimol at concentrations of up to 10^{-4} M (NOEC = >100 µM), and hence had no affinity for GABA-A receptors.

Hippocampus slice test.

FIN showed no excitatory or convulsive potential in this sensitive model (excitatory potential; 98.2% ± 5% of control), confirming its lack of affinity for GABA-A receptors . In contrast, TVO showed an effect (excitatory potential; 276% ± 31% of control) (Figure 5).

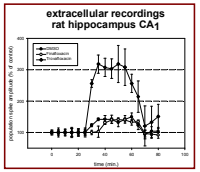

Figure 5. Effect of FIN and TVO on electrically stimulated rat hippocampus slices.

Literature

[1] Wohlert *et al.*, 48th ICAAC, Washington DC 2008, Poster No. F1-2036.
[2] Kresken *et al.*, 48th ICAAC, Washington DC 2008, Poster No. F1-2037.
[3] Goh *et al.*, 48th ICAAC, Washington DC 2008, Poster No. F1-2042.
[4] Endermann *et al.*, 48th ICAAC, Washington DC 2008, Poster No. F1-2044.
[5] Endermann *et al.*, 48th ICAAC, Washington DC 2008, Poster No. F1-2045.
[6] Patel *et al.*, 48th ICAAC, Washington DC 2008, Poster No. F1-2048.

Conclusions

- FQs suffer from a number of toxic effects, some of which have led to compounds being withdrawn. The test systems used here are all well validated *in vitro* or *ex vivo* systems designed to test possible toxic effects.
- Juvenile dogs have been shown to be more susceptible than rodents to chondrocyte toxicity which is associated with FQ-induced arthropathy. CIP displayed toxicity against the dog and human cell lines whereas the lack of effect with FIN in this model is promising.
- Hepatotoxic, chondrotoxic, phototoxic and neurotoxic potential were determined at concentrations well above those detected in human plasma following dosing [6]. FIN had no effects in these test systems whereas CIP and TRO did display toxicity.
- Compounds inhibiting the hERG channel have been shown to prolong the cardiac action potential and hence the QT interval in man. FIN had no effect in this test (unlike SPA).
- These findings (summarised in Figure 6) taken in conjunction with the excellent tolerability by the oral route in Phase I studies in man [6] and the good activity in animal infection models [4, 5] indicate that finafloxacin is an excellent candidate for progression to the clinic.

Figure 6. Summary of the assessment of FIN in a panel of predictive *in vitro* and *ex vivo* toxicity assays

Test system	Finafloxacin result	Comparator result
Cytotoxicity mouse Mϕ J774.A1	EC_{50} = 100 µg/mL	EC_{50} = 80 µg/mL (CIP)
Phototoxicity mouse fibroblasts 20'/1h radiation	EC_{50} > 100 µg mL	EC_{50} > 100 / = 60 µg/mL (CIP)
Hippocampus slice test	98 % of control	276 % of control (TRO)
Primary hepatocytes	NOEC ≥ 100 µg/mL	NOEC 10 µg/mL (TRO)
Primary dog chondrocytes	NOEC ≥ 100 µg/mL	NOEC = 10 µg/mL (CIP)
Primary human chondrocytes	NOEC ≥ 100 µg/mL	NOEC = 30 µg/mL (CIP)
GABA-A receptor	NOEC > 100µM	0.3 µM ((+)-bicuculline)
Langendorff heart preparation	NOEC > 300 µM	-
hERG channel activity	NOEC > 300 µM	ED_{50} ~ 25 µM (SPA)