50% Effective dose (ED50) Determination of KPI-10 for Treating Sepsis in Mice Due to Fluoroquinolone-Susceptible (FQ-S) and Fluoroquinolone-Resistant (FQ-R) Nosocomial Pathogens: Comparison with Ciprofloxacin (CIP)

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Abstract

Background: KPI-10 is a next generation fluoroquinolone (FQ) with potent activity against multidrug-resistant pathogens, including FQ-R strains. Using the murine sepsis model, the aim of the study was to determine the *in vivo* efficacy of KPI-10 in comparison with CIP against selected FQ-S and FQ-R gram-positive and gram-negative organisms.

Methods: The MICs for KPI-10 and CIP for *E. coli* FQ-S, *E. coli* FQ-R, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, *S. aureus* (MRSA), Group A *Streptococcus*, and *S. pneumoniae* strains are summarized in the Table. Mice were infected intraperitoneally with 0.5 mL of the bacterial inoculum (LD100). Treatment was injected subcutaneously 1 and 4h after the bacterial challenge. For each drug, concentrations ranged from 0.01 to 100 mg/kg. After an observation period of 5 days, the Reed and Muench method was used for the determination of the ED50.

Results: See Table 1 and Table 2.

Conclusion: KPI-10 exhibited similar *in vivo* activity to CIP against FQ-S strains; however, KPI-10 demonstrated superior *in vivo* activity against FQ-R Gram-negative isolates, including *E. coli* and *A. baumannii*. KPI-10 also demonstrated excellent activity against MRSA, with a 14-fold lower ED50 compared with that of CIP. Despite a low MIC for KPI-10, the KPI-10 ED50 associated with *S. pneumoniae* was similar to that of CIP. The results indicated that KPI-10 is a highly potent and effective antibacterial agent against both gram-positive and gram-negative pathogens in the murine sepsis model, including FQ-R organisms. KPI-10 is a promising new agent for the treatment of infections due to emerging resistant pathogens.

Background

Fluoroquinolones (FQs) are a family of synthetic, broad-spectrum antibiotics typically known for their excellent activity against Gram-negative pathogens. These antibiotics demonstrate good activity against many pathogens involved in pneumonia and urinary tract infections, but rising resistance has left existing FQs less useful against more resistant bacteria.
KPI-10 is a next-generation FQ with potent activity against Gram-negative and Gram-positive multidrug-resistant pathogens, including FQ-R strains.

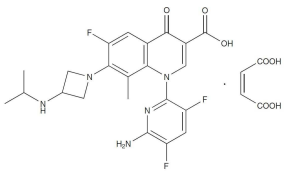


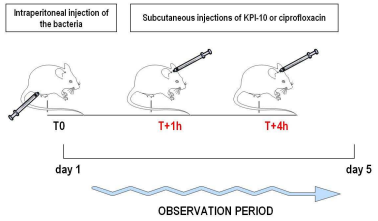
Figure 1. **Structure of KPI-10**

Materials & Methods

Bacterial strains. Gram-negative and Gram-positive key pathogens were used in this study (isolated in Nantes, Hotel-Dieu Hospital, France):
1. Group A *Streptococcus*, FQ-S strain
2. *Streptococcus pneumoniae*, FQ-R strain
3. Methicillin-resistant *S. aureus* (MRSA), FQ-R strain
4. *Escherichia coli*, FQ-S and FQ-R strains
5. *Acinetobacter baumannii*, FQ-R strain
6. *Pseudomonas aeruginosa*, FQ-S strain
7. *Klebsiella pneumoniae*

Antibiotics. KPI-10 (Lot #: 101004) was provided by Kalidex Pharmaceuticals Inc. Clinical forms of ciprofloxacin and levofloxacin were used and supplied by the respective manufacturers. The preparation of dose solution was performed according to the procedures provided by the manufacturers.

MICs determination. The MICs of KPI-10, ciprofloxacin, and levofloxacin were determined in cation-supplemented MH broth by the CLSI microdilution technique (1). Overnight MH broth cultures were used to prepare inocula of 10^5 CFU/mL. The MIC was defined as the lowest concentration of antimicrobial agent that prevented turbidity after 24 h of incubation at 37°C.



Animal model. Six-week-old pathogen-free mice (weight, 20-24g) were used for this study (Janvier, France). Bacterial cells from overnight cultures were collected by centrifugation (2500 rpm, 10 min.), washed two times using sterile saline 0.9%, and then appropriately diluted suspensions of the infecting inoculum were prepared using 5% porcine gastric mucin (Sigma®, St. Louis, Mo, USA). Mice (20-24g) were infected intraperitoneally with 0.6 mL of appropriately diluted suspensions of the infecting inoculum. Half-log dilutions of studied drugs (KPI-10 or ciprofloxacin) were prepared in sterile saline (0.9%) from 0.01 mg/kg to 200 mg/kg. Treatment was administered by subcutaneous injection at 1 and 4 hours after bacterial challenge. Mice were observed for up to 5 days for mortality. The method of Reed and Muench (cumulative distribution function) was used for the determination of the ED50, as previously described (2).

Results

• MICs of KPI-10, ciprofloxacin (CIP), and levofloxacin (LVX) for tested isolates are summarized in the Table 1.

• ED50 values of KPI-10 and ciprofloxacin (CIP) for Gram-positive isolates and Gram-negative isolates are shown in Table 2.

Table 1. MICs of KPI-10, ciprofloxacin (CIP), and levofloxacin (LVX) for tested isolates.

STRAIN			MIC (mg/L) KPI-10	CIP	LVX
S. aureus	FQ-R	SA BCB8	1	16	4
S. pneumoniae	FQ-R	SP 66	0.06	2	4
Group A Strep	FQ-S	SP 10	0.06	1	0.5
E. coli	FQ-S	EC 19	0.03	0.015	0.03
	FQ-R	EC 32	2	16	16
K. pneumoniae	FQ-R	KPN 6609	4	32	32
A. baumannii	FQ-R	AB46	1	32	8
P. aeruginosa	FQ-S	PA C10	0.015	0.0075	0.03

Table 2. 50% effective dose (mg/kg) for KPI-10 and ciprofloxacin (CIP) against key pathogens.

STRAIN			ED50 (mg/kg) KPI-10	CIP
S. aureus	FQ-R	SA BCB8	5.1	71.4
S. pneumoniae	FQ-R	SP 66	15.7	17.3
Group A Strep	FQ-S	SP 10	0.1	0.06
E. coli	FQ-S	EC 19	0.4	0.2
	FQ-R	EC 32	0.9	51.0
K. pneumoniae	FQ-R	KPN 6609	1.0	11.2
A. baumannii	FQ-R	AB46	1.2	49.5
P. aeruginosa	FQ-S	PA C10	0.8	0.2

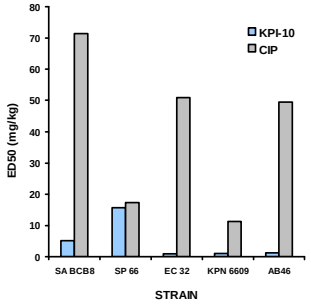


Figure 2. In vivo activity of KPI-10 and ciprofloxacin (CIP) against FQ-R isolates.

Discussion / Conclusions

• KPI-10 was associated with excellent in vivo bactericidal activity against E. coli, A. baumannii, P. aeruginosa, and MRSA. The ED50 determined for both FQ-S E. coli and FQ-S P. aeruginosa are in correlation with in vitro data (E. coli, 0.4/0.2 mg/kg and P. aeruginosa, 0.8/0.2 mg/kg for KPI-10 and ciprofloxacin, respectively).

• For more challenging FQ-R Gram-negative isolates, KPI-10 showed significantly lower ED50 with values around 1 mg/kg (from 0.9 to 1.2 mg/kg) compared to those of ciprofloxacin. The ED50 of KPI-10 against both FQ-R E. coli and FQ-R A. baumannii was much lower when compared with ciprofloxacin: KPI-10 appeared to be between 40- and 60-fold more active than ciprofloxacin against these resistant isolates. The similar observation was made against a FQ-R K. pneumoniae strain but at a less extent (KPI-10 10-fold more active than ciprofloxacin).

• **In the murine sepsis model using the ED50 as the endpoint, KPI-10 demonstrated promising in vivo activity against nosocomial resistant pathogens, including FQ-R E. coli, FQ-R A. baumannii, and FQ-R MRSA. Further studies using discriminative animal experimental models are considered to confirm the superiority of KPI-10 over other FQs against key Gram-negative and Gram-positive key pathogens.**

Acknowledgement

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(2) Reed, L.J., and H. Muench. 1938. A simple method of estimating fifty percent endpoints. Am. J. Hyg. 27:493-497

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