Demonstration of the Broad-Spectrum In Vitro Activity of Finafloxacin against Pathogens of Biodefense Interest

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ABSTRACT This study investigated the in vitro activity of finafloxacin against bacterial strain panels of the biodefense pathogens. Broth microdilution assays were performed at neutral and acidic pH to determine the effectiveness of the antibiotics under conditions typical of an intracellular environment. In all instances, finafloxacin demonstrated superior activity at low pH. These results highlight the importance of evaluating antimicrobial efficacy under conditions relevant to those encountered in vivo.

KEYWORDS finafloxacin, in vitro activity, acidic pH, biothreat pathogens, acid environments, biodefense, in vitro

Antimicrobial resistance is an evolving issue, and new therapeutics are needed to treat infections caused by the pathogens of biodefense interest and those that are considered to be of public health concern. It is important that new antimicrobials are evaluated under conditions that model those encountered within the environment of a host, including the low-pH environment of the cell (the phagolysosome) that is particularly relevant to intracellular pathogens and infected body sites. It has been shown previously that the activity of certain classes of antibiotics (including fluoroquinolones) can be affected by a reduction in pH (1–4). Finafloxacin is a fluoroquinolone derivative with an 8-cyano substituent and 7-pyrrolo-oxazinyl moiety that is being developed for the treatment of urinary tract infections in hospitalized patients (5, 6). This modification has conferred activity in low-pH environments, which has resulted in superior in vitro activity against a range of organisms, including Staphylococcus aureus and Acinetobacter baumannii (7, 8).

The availability of formulations of finafloxacin that can be delivered orally and systemically makes finafloxacin a worthy alternative for the treatment of a range of infections. In addition to good safety and efficacy data obtained in patients suffering from complicated urinary tract infections and pyelonephritis, previous studies have also demonstrated efficacy against the bioterror agents Burkholderia pseudomallei and Francisella tularensis in vitro and in vivo (6, 9–11). The aim of this study was to further evaluate the in vitro activity of finafloxacin against larger strain panels of biodefense pathogens.

Antibiotic susceptibility was determined at pH 5 and pH 7 for B. pseudomallei (n = 10), Burkholderia mallei (n = 10), F. tularensis (n = 10), Bacillus anthracis (n = 10), and Yersinia pestis (n = 10), held at the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) (Table 1). In addition, a B. pseudomallei strain panel (n = 11) provided by the Biomedical Advanced Research and Development Authority (BARDA) was screened (Table 1) (12). These assays were performed under biosafety level 3 (BSL3) conditions. Antibiotic susceptibility was reported as the MIC 50 or MIC 90, defined as the lowest concentration of the antibiotic at which the growth of 50% or 90% of the isolates, respectively, were inhibited.

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TABLE 1 Panel of bacterial strains evaluated

| Organism     | Strain* | Origin | Source |
|--------------|---------|--------|--------|
| B. pseudomallei |         |        |        |
|              | 316c    | Thailand | Human |
|              | E203    | Thailand | Unknown |
|              | NCTC4845 | Singapore | Monkey |
|              | STW115-2 | Thailand | Water |
|              | STW199-2 | Thailand | Water |
|              | E8      | Thailand NE | Soil |
|              | PS2237  | Vietnam | Unknown |
|              | WRAIR1188 | Malaysia | Human |
|              | K96243  | Thailand | Human |
|              | 1026b   | Thailand | Human |
|              | K96243* | Thailand | Human |
|              | 1026b*  | Thailand | Human |
|              | HBPUB10134A* | Thailand | Human |
|              | HBPUB10303A* | Thailand | Human |
|              | 1106a*  | Thailand | Human |
|              | MSHR 305* | Australia | Human |
|              | MSHR 668* | Australia | Human |
|              | MSHR 5855* | Australia | Human |
|              | MSHR 5848* | Australia | Human |
|              | MSHR 5858* | Thailand | Human |
|              | 406e*   | Thailand | Human |
| F. tularensis |         |        |        |
|              | LVS     | Former Soviet Union | Water rat |
|              | OR01-1807 | USA | Unknown |
|              | FRAN003  | USA | Unknown |
|              | FRAN005  | USA | Unknown |
|              | FRAN006  | USA | Unknown |
|              | FRAN007  | USA | Unknown |
|              | FRAN012  | USA | Unknown |
|              | FRAN013  | USA | Unknown |
|              | FRAN016  | USA | Unknown |
|              | SCHUS4-1 | USA | Human |
| B. anthracis |         |        |        |
|              | Vollum1B | USA | Bovine |
|              | Sterne   | South Africa | Bovine |
|              | Ames     | USA | Bovine |
|              | K1938    | Indonesia | Unknown |
|              | K5926    | India | Unknown |
|              | K7038    | South Korea | Unknown |
|              | SK57     | England | Unknown |
|              | K7978    | Namibia | Unknown |
|              | Africa33 | South Africa | Unknown |
|              | K8091    | Norway | Unknown |
| B. mallei    |         |        |        |
|              | GB3 (ATCC 120) | UK | Unknown |
|              | GB4      | Turkey | Human |
|              | GB5      | Hungary | Unknown |
|              | GB6      | Turkey | Human |
|              | GB7      | Turkey | Unknown |
|              | GB8 (China7) | Burma | Human |
|              | GB9      | India | Mule |
|              | GB10     | India | Horse |
|              | GB11     | China | Horse |
|              | GB12     | Hungary | Unknown |
| Y. pestis    |         |        |        |
|              | CO92     | USA | Human |
|              | C12      | USA | Human |
|              | antiqua  | Congo | Human |
|              | pestoidesB | Former Soviet Union | Human |
|              | pestoides Fmp1 | Former Soviet Union | Human |
|              | Yeo154   | Japan | Human |
|              | Angola   | Angola | Human |
|              | Java9    | Indonesia | Human |
|              | M111(74) | Madagascar | Human |
|              | LaPaz    | Bolivia | Human |

*Strains with an asterisk belong to the BARDA strain panel. All other strains were obtained from the USAMRIID Unified Culture Collection (UCC), Frederick, MD, USA.
Finafloxacin was supplied by MerLion Pharmaceuticals GmbH, and all other antibiotics were sourced from the U.S. Pharmacopeia, Selleckchem, or Sigma. Broth microdilution assays were performed as detailed by the Clinical and Laboratory Standards Institute (CLSI) (13), with the exception of a medium supplement (2%), IsoVitaleX (Becton, Dickinson), used to support the growth of \textit{F. tularensis}. The activity of finafloxacin was determined at pH 5 and pH 7 (if the bacterial species was able to be cultured) and the MICs determined.

At pH 5, the MICs for \textit{B. pseudomallei} ranged from 0.12 to 2 µg/ml, 16 to 64 µg/ml, and 4 to 64 µg/ml for finafloxacin, ciprofloxacin (CIP), and ceftazidime (CAZ), respectively, demonstrating the superior \textit{in vitro} activity of finafloxacin at low pH. Although it is difficult to make comparisons between the efficacies of antibiotics simply by MIC, these values are lower than those determined for another fluoroquinolone, CIP, and a component of the treatment for \textit{B. pseudomallei} infections in humans (CAZ) (Fig. 1A). At neutral pH, finafloxacin demonstrated a level of activity (0.5 to 8 µg/ml) similar to those observed with CIP (1 to 4 µg/ml) and CAZ (0.5 to 32 µg/ml) (Fig. 1B).

A similar trend was observed with the other pathogens of biodefense interest. Finafloxacin had superior activity at pH 5 for \textit{B. anthracis}, \textit{B. mallei}, and \textit{Y. pestis} compared to either CIP or azithromycin (AZM) (an antibiotic used for the treatment of \textit{B. mallei} infection in humans and as a control in the \textit{in vitro} assays) (Table 2). Unfortunately, only two strains of \textit{F. tularensis} could be cultured in this low-pH environment; therefore, the MIC$_{50}$ and MIC$_{90}$ at pH 5 could not be determined. The most striking difference was observed for \textit{B. mallei}. Finafloxacin had 9-fold and 7-fold improved activity over that of AZM against a panel of these strains (MIC$_{50}$, 0.12 µg/ml compared to >64 µg/ml; MIC$_{90}$, 0.5 µg/ml compared to >64 µg/ml) when performed at pH 5 (Table 2). At pH 7, finafloxacin demonstrated activity similar to those of the comparator antibiotics, with MIC$_{50}$ and MIC$_{90}$ of 0.5 µg/ml (at both pHs) for \textit{B. mallei} and 0.06 µg/ml and 0.12 µg/ml for \textit{B. anthracis}, respectively (Table 2).

The data set detailed in these studies demonstrates that finafloxacin has activity under both acidic and neutral conditions, with enhanced activity of finafloxacin in low-pH environments, where other antibiotics (including ciprofloxacin) have reduced activity. This has been demonstrated for all of the biodefense pathogens of interest and is in agreement with data generated by other groups (7, 8, 10, 11). The improved activity of finafloxacin compared to that of ciprofloxacin (a typical treatment for infections caused by \textit{B. anthracis}, \textit{Y. pestis}, and \textit{F. tularensis}) further highlights the importance of evaluating therapies under conditions considered to be more like those encountered within a host and identifies finafloxacin as a novel broad-spectrum fluoroquinolone that could be used for prophylaxis or treatment following exposure to a range of pathogens.

Of particular interest is the activity of finafloxacin against the \textit{Burkholderia} species evaluated. It has been demonstrated previously that fluoroquinolones are not effective as treatment for melioidosis in humans mainly due to \textit{B. pseudomallei} possessing resistance mechanisms, including efflux pumps (14–17). The results detailed in this

**FIG 1** Cumulative MICs determined for a panel of \textit{B. pseudomallei} strains for finafloxacin (\textit{n} = 21), ciprofloxacin (\textit{n} = 11), and ceftazidime (\textit{n} = 11) at pH 5 (A) and pH 7 (B).

Finafloxacin was supplied by MerLion Pharmaceuticals GmbH, and all other antibiotics were sourced from the U.S. Pharmacopeia, Selleckchem, or Sigma. Broth microdilution assays were performed as detailed by the Clinical and Laboratory Standards Institute (CLSI) (13), with the exception of a medium supplement (2%), IsoVitaleX (Becton, Dickinson), used to support the growth of \textit{F. tularensis}. The activity of finafloxacin was determined at pH 5 and pH 7 (if the bacterial species was able to be cultured) and the MICs determined.

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| Species     | MIC<sub>50</sub> (µg/ml) by pH<sup>a</sup> | MIC<sub>90</sub> (µg/ml) by pH<sup>a</sup> | Range (µg/ml) by pH<sup>a</sup> |
|-------------|----------------------------------------|----------------------------------------|---------------------------------|
|             | pH 5        | pH 7        | pH 5        | pH 7        | pH 5        | pH 7        | pH 5        | pH 7        | pH 5        | pH 7        |
|             | FIN CIP AZM | FIN CIP AZM | FIN CIP AZM | FIN CIP AZM | FIN CIP AZM | FIN CIP AZM | FIN CIP AZM | FIN CIP AZM | FIN CIP AZM | FIN CIP AZM |
| B. anthracis| 0.03        | 0.06        | ND          | 0.03        | 0.06        | ND          | 0.12        | 0.03        | ND          | 0.06        | 0.12        | 0.06 to 0.12 | 0.03 to 0.06 | ND          |
| B. mallei   | 0.12        | ND          | >64         | 0.5         | ND          | 0.25        | 0.5         | ND          | >64         | 0.5         | ND          | 0.06 to 0.5  | 0.03 to 0.5  | ND          |
| Y. pestis   | =0.03       | 0.5         | ND          | =0.03       | 0.015       | ND          | 0.06        | 0.03        | ND          | =0.03       | 0.12 to 1   | =0.03 to 0.12 | 0.008 to 0.03 | ND          |
| F. tularensis | ND          | ND          | ND          | =0.03       | 0.015       | ND          | ND          | ND          | ND          | =0.03       | 0.008 to 0.25 | ND          |

<sup>a</sup>ND, not determined.
communication suggest that finafloxacin is not affected by the efflux pumps in \*B. pseudomallei\* that confer resistance to other fluoroquinolones, possibly due to the effect of the modified chemical structure (7, 10, 18). The promising data generated for \*B. mallei\* suggest that finafloxacin is a potential alternative for the treatment of infection caused by this organism.

Finafloxacin appears to have a wider spectrum of activity than the other fluoroquinolones and has the potential to be used to treat infections caused by all of the bioterror pathogens evaluated (19). It has also been shown to be safe and well tolerated in clinical trials (6). These encouraging \textit{in vitro} findings warrant further investigation of finafloxacin which would determine whether this activity translates into comparable protection against all of these pathogens \textit{in vivo}.

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