Antibacterial activities of iron chelators against common nosocomial pathogens

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Running Title: Iron chelator activity against nosocomial bacteria

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Abstract

The activity of iron chelators (deferoxamine, deferiprone, Apo6619, and VK28) was evaluated against type strains of *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Escherichia coli*. Deferiprone, Apo6619, and VK28 each inhibited growth in standard and RPMI tissue culture medium, while deferoxamine had no effect. Additionally, time-kill assays revealed that VK28 had a bacteriostatic effect against *S. aureus*. Therefore, these newly developed iron chelators might provide a non-traditional approach to treat bacterial infections.
Iron is an essential cofactor of biochemical pathways in both prokaryotic and eukaryotic species. Numerous studies have assessed the potential viability of iron chelators as therapeutic agents against various microbes with only mixed success (4, 6-8, 10-17 21, 24, 25, 27). Nevertheless, as novel iron chelators are developed for treatment applications such as neurodegenerative diseases (3, 9, 18) or β-thalassemia (3, 9), the evaluation of their antimicrobial activity should be tested because their efficacy against bacteria may be superior to chelators previously tested. In the case of multidrug-resistant (MDR) bacteria, where entire classes of antibiotics are no longer treatment options (1, 20), iron chelators that have already undergone toxicity and preclinical testing in animals might provide an alternative treatment approach. MDR-bacteria such as Staphylococcus aureus or Acinetobacter baumannii are exceedingly difficult to treat because of nosocomial spread and infections in immunocompromised patients (1, 18, 20). The same microbes have also been responsible for wound infections incurred by military personnel that are often immunocompromised after polytrauma (2, 23).

Defersirox and deferoxamine are approved by the U.S. Food and Drug Administration (FDA), but have demonstrated limited efficacy in combating bacterial infection (4, 6, 16). Deferoxamine is a siderophore, a molecule secreted by bacteria to capture iron; therefore, many bacteria challenged with deferoxamine also harbor a receptor capable of capturing such molecules when complexed to iron (4, 6, 17). Defersirox, while rationally designed to bind iron, failed to treat fungal infections (21) and is considered toxic (11).

In this study, we sought to assess the antibacterial effects of iron chelators that have yet to be tested against bacteria, as well as deferiprone, a chelator recently FDA-approved for iron
overload due to blood transfusions in patients with thalassemia. Deferiprone has also been shown to have antibacterial properties against certain bacterial species in vitro (4, 10). Deferiprone (ApoL1) and Apo6619 were provided by ApoPharma, Inc., and VAR10100 (VK28 dihydrochloride) was provided by Varinel, Inc. Because of their iron chelation properties, both Apo6619 and VK28 (and their derivatives) are currently being studied for treatment applications (9, 19, 22, 28). Deferoxamine mesylate salt (DFO) and 2,2'-Bipyridyl (DIP) were purchased from Sigma-Aldrich Inc. and were evaluated for comparison purposes.

Bacterial type strains considered common nosocomial infectious agents were acquired from the American Type Culture Collection (ATCC): A. baumannii (19606, 17978), Pseudomonas aeruginosa (PAO1, 27853), S. aureus (43300, 25923), Klebsiella pneumoniae (BAA-2146, 700603), and Escherichia coli (35718, 43888). The MIC of DIP, DFO, ApoL1, Apo6619, and VK28 were determined by following the microdilution methodology recommended by the CLSI (5) in cationic-adjusted Mueller-Hinton Broth (CAMHB) against the bacteria listed above. The MIC was also determined in RPMI 1640 tissue culture media (Life Technologies, Inc.), which may better represent the human host environment with limited amounts of cofactors such as calcium, magnesium, zinc, and iron. Time-kill assays against S. aureus and E. coli were performed as described by White, et al. (24). An initial inoculum of ~1.0 x 10^7 CFU/mL was challenged with either 1X or 2X MIC of VK28, and cells were grown at 37°C for 24 hours. Samples were taken at 0, 2, 6, and 24 hours and the CFU/mL was determined via dilution and plating with a spiral plater (Advanced Instruments, Inc.) where each sample was diluted over three logs onto plates containing CAMHB media and agar. Biological replicates for all tests were performed at least three times in triplicate (technical replicates).
Results and Discussion

DFO did not affect bacterial growth in CAMHB (MIC>512 µg/mL for all bacteria tested) (Table 1). The result was not surprising since the compound may readily deliver iron to bacteria with a cognate siderophore receptor. In contrast, VK28 inhibited the growth of *A. baumannii*, *E. coli* and *S. aureus* in CAMHB. Further, both ApoL1 and Apo6619 inhibited the growth of some strains of *P. aeruginosa*, *K. pneumoniae*, as well as *E. coli* and *A. baumannii*, while no effect on *S. aureus* was observed (Table 1). Because CAMHB is a rich broth with excess iron, carbon sources, and other cofactors far exceeding the levels in the human body, RPMI medium was chosen to evaluate the activity on the same bacterial strains in a more restrictive media. When challenged with the iron chelators that showed the most promise in CAMHB, the MIC was reduced accordingly in this co-factor-limited environment (Table 2). When evaluated in RPMI, VK28 the MIC improved 4 to 64-fold, and ApoL1 and Apo6619 improved 2 to 4-fold (Table 2).

Previous studies with iron chelators have demonstrated a bacteriostatic effect on bacterial growth (8, 13, 15). We performed a time-kill assay to see if this was also true of the iron chelators evaluated in this study. *E. coli* and *S. aureus* were both challenged with either 1X or 2X of the determined MIC for VK28 (Figure 1). Growth of *S. aureus* was attenuated somewhat by both concentrations (Figure 1A). Additionally, VK28 proved to have a bacteriostatic effect on *E. coli* at 2X the MIC (Figure 1B). Similar bacteriostatic effects were observed for ApoL1 and Apo6619 (data not shown).

Unlike the mild effects observed with DFO and other iron chelators in previous studies (4, 9), VK28, ApoL1, and Apo6619 had pronounced effects on the growth of nosocomial bacteria. The outcomes observed could be related to the rational design of these iron chelators.
For example, VK28 includes a piperazine ring which enhances polarity to cross the blood brain barrier for the treatment of neurodegenerative disease (18, 28). It is possible that this polarity may also allow the chelator cross certain bacterial membranes. ApoL1, in contrast, is a very small, neutral molecule, and these properties are known to facilitate its passage across host cell membranes and perhaps bacterial membranes as well. Therefore, in each case, the free iron might be chelated both inside and outside the bacteria explaining enhanced efficacy. Continued studies on these molecules, including combinatorial therapy with conventional antibiotics and animal modeling, will attempt to uncover the mechanisms by which these iron chelators provide a potent antimicrobial effect.
Acknowledgements

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Table 1 – MIC of iron chelators against ATCC type strains grown in CAMHB.

<table>
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<tr>
<th>Bacterial Species</th>
<th>Strain Number</th>
<th>MIC of Iron Chelators (ug/mL)</th>
<th>DIP</th>
<th>DFO</th>
<th>ApoL1</th>
<th>Apo6619</th>
<th>VK28</th>
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<td>&gt;512</td>
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Table 2 - MIC of iron chelators against ATCC type strains grown in RPMI1640 media. N/A means not attempted.

<table>
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<tr>
<th>Bacterial Species</th>
<th>Strain Number</th>
<th>MIC of Iron Chelators (ug/mL)</th>
<th>DIP</th>
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Figure 1 – Time-kill studies of VK28 against *Staphylococcus aureus* 43300 (A) and *Escherichia coli* 48333 (B). No treatment represents growth in the absence of chelator. Detection limit is 1.0 x 10^3 CFU/mL.
Figure 1

A

B