Novel Cloudbreak Bifunctional Molecule Protects Against *Acinetobacter* Pneumonia

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ABSTRACT

Background: Multidrug-resistant (MDR) organisms due to Gram-negative bacilli (GNB), including those related to *Acinetobacter baumannii* (*AB*) have become endemic in healthcare systems throughout the world. Novel methods to treat these infections are needed. Septicemia triggered by lipopolysaccharide (LPS) is a common manifestation of GNB infections that results in vascular leak, tissue edema, organ failure, and death. The bifunctional Cloudbreak molecule, Compound A (Cpd-A), consists of a Targeting Moiety (TM) joined to an Effector Moiety (EM) via a stable linker. The TM is a small molecule which binds LPS while the EM is an Fc domain of human IgG1 to engage host Fcγ and FcRn receptors. We evaluated the activity of Cpd-A against *AB in vitro* and *in vivo*.

Materials/methods: A trans-well permeability assay was used to study the role of Cpd-A in enhancing vascular integrity of human umbilical vein endothelial cells (HUVECs) infected with *AB*. Cpd-A was evaluated for efficacy in protecting against *AB* pneumonia (due to MDR strain HUMC1) using neutropenic mice (cyclophosphamide [200 mg/kg] and cortisone acetate [500 mg/kg] on day -2, +3, and +8 relative to infection). Treatment with 10 mg/kg Cpd-A started 3 h post infection and continued through Day +7 daily (qd) or every other day (qod) and was compared to colistin treatment (2.5 mg/kg bid, qd). Endpoints were mouse survival by Day +21 and lung CFU by Day +4.

Results: Cpd-A at 0.1, 0.5, and 1.0 μM reduced trans-membrane HUVECs leakage caused by *AB* by ~50-85% (P<0.0001). Further, treatment of mice with Cpd-A resulted in enhanced survival comparable to colistin treatment versus placebo (40% and 70% for Cpd-A qd and qod, respectively, 60% for colistin, vs. 0% survival for placebo p<0.03 for all treatments). Finally, Cpd-A qd treatment resulted in almost complete sterilization of lungs, while Cpd-A qod or colistin qd treatment resulted in 4- and 3-log reduction of lung bacterial burden, respectively (P<0.0001).

Conclusions: Cpd-A has potent activity against *AB* mouse infection possibly by a dual killing mechanism and by reducing LPS-induced vascular leak. Continued investigation of Cpd-A as a novel treatment for MDR-GNB is warranted.

INTRODUCTION/AIMS

- MDR GNB septicemia, including those caused by *AB,* is a predominant cause of healthcare-associated infections with high mortality rates (1).
- These infections are treated with highly toxic antibiotics and sometimes are untreatable due to drug pan resistance (1).
- Recent data showed that LPS triggers a TLR-4-mediated activation of the MyD88/NF-kB cascade which leads to robust inflammatory immune response (2, 3).
- LPS also triggers a MyD88/ARF-GTP activation pathway that leads to vascular leak and ultimate organ failure (4).
- Cpd-A (Cloudbreak, Cidara) consists of a Targeting Moiety (TM) joined to an Effector Moiety (EM) via a stable linker.
- The TM binds LPS while the EM is an Fc domain of human IgG1 to engage host Fcγ and FcRn receptors.
- We sought to determine the role of Cpd-A in protecting against *AB*-mediated vascular leak *in vitro* and in protecting against murine *AB* penumonia.

METHODS

Endothelial cells, bacterial strains, inhibitors and biological materials. Human umbilical vein endothelial cell (HUVEC) were used. A virulent clinical isolate *AB* HUMC1 (a multidrug resistant [MDR] isolate with susceptibility to only colistin) was used. *AB* HUMC1 is known to produce and shed LPS. Cpd-A was provided by Cidara Therapeutics.

Permeability assay. HUVECs were seeded on transwell inserts prior to challenging them with *AB* HUMC1 strain (10^7 cells) with or without different concentrations of Cpd-A in phenol red-free M-199 medium. Three hours following the incubation period, FITC-dextran-10K was added to the top chamber and vascular leak was determined by quantifying the concentration of the dye in the bottom chamber by florescence microplate reader 1 h after addition.

Mouse model. CD-1 mice were immunosuppressed with cyclophosphamide (200 mg/kg) and cortisone acetate (500 mg/kg) on days -2, +3, and +8, relative to infecting them with aerosolized *AB* HUMC1 (5). Treatment with Cpd-A (10 mg/kg, qd; or 10 mg/kg, qod) was given i.p. 3 hours post infection and continued through Day +7 days, relative to infection. Mice treated with colistin (2.5 mg/kg, bid) through Day +7. Survival of mice was followed through Day +21. In a seperate experiment, immunosuppressed mice were infected and treated as above and lungs bacterial burden on Day +4 was evaluated.

Statistical Analysis. Differences in HUVEC permeability, and lung CFU were compared by the nonparametric Wilcoxon rank-sum test. The nonparametric log-rank test was used to determine differences in survival times. A *P* value < 0.05 was considered significant.

SUMMARY/CONCLUSIONS

- Cpd-A reduced HUVECs vascular permeability in a dose dependent manner.
- Cpd-A protected mice from pneumonia induced by MDR *AB*. This protection was comparable to the standard treatment afforded by colistin.
- Cpd-A qod treatment resulted in better mice survival than qd treatment despite the almost complete sterilization of the lungs by the qd treatment.
- Cpd-A has potent activity against *AB* mouse infection possibly by a dual killing mechanism and by reducing LPS-induced vascular leak.
- Continued investigation of Cpd-A as a novel treatment for MDR-GNB is warranted

RESULTS

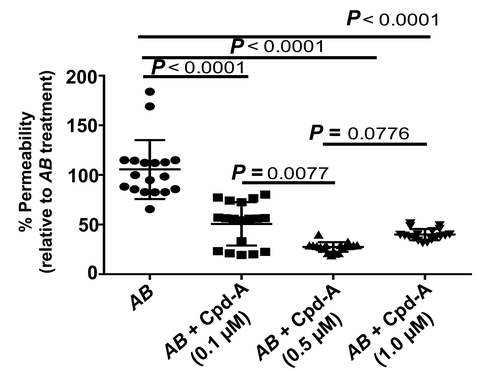

Figure 1. Cpd-A protects HUVECs from *AB*-induced vascular permeability *in vitro*. Permeability assays of HUVECs were conducted in the presence of 10^7 cell of *AB* HUMC1 with or without different concentrations of Cpd-A.

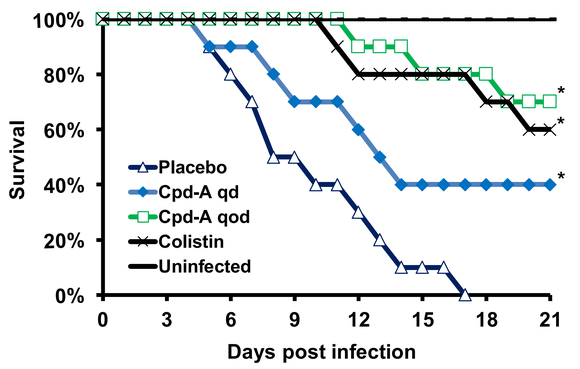

Figure 2. Cpd-A prolongs survival of neutropenic mice with *AB* pneumonia. Survival of mice (n=10 per group except for uninfected controls which had 5). **P*<0.03 vs. placebo by Log Rank test.

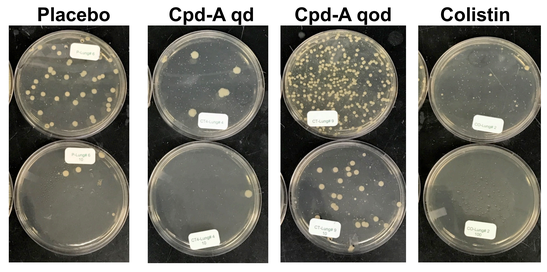

Figure 3. Representative plates showing Cpd-A activity versus placebo or colistin in affecting *AB* lung burden. Neutropenic mice were infected and treated with Cpd-A qd, Cpd-A qod, or colistin. Mice were sacrificed on Day +4, relative to infection and their lungs harvested, homogenized and cultured. The representative plates are from undiluted cultures showing almost complete sterilization with Cpd-A qd treatment.

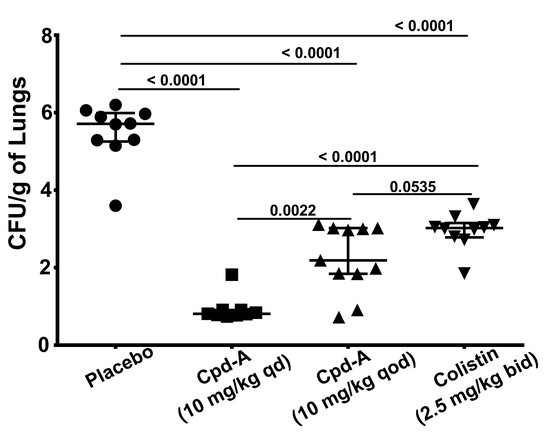

Figure 4. Cpd-A is effective in lowering lungs *AB* burden. Neutropenic mice (n=10 or 11 per group) were infected and treated with Cpd-A qd, Cpd-A qod, or colistin and on Day +4 were sacrificed and their lungs quantitatively cultured. While cpd-A qd treatment almost completely sterilized the lungs, cpd-A qod was as effective as colistin in resulting in resulting in 3-4 log reduction compared to placebo-treated mice.

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ACKNOWLEDGEMENTS

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