Research Abstract

Elucidating the mechanism of action of novel polymer-based synthetic anti-infective compound RECCE® 327

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Abstract

Antimicrobial resistance (AMR) is an urgent global health threat. Multi-drug resistant bacteria are on the rise and have outpaced the development of effective antibiotics threatening our ability to treat common infections. Currently, the global antibiotic pipeline remains deficient as most drugs advancing through the clinic are predominantly derivatives of well-established antibiotic classes from more than 30 years ago and do not include any new class of molecules. We investigated the potential mechanism of action of a new synthetic polymer anti-infective with rapid and potent broad-spectrum bactericidal activity known as RECCE® 327 (R327). We evaluated the bactericidal activity of R327 and determined to what extent this activity was reversible in wild type *E. coli*. We explored R327's impact on cellular bioenergetics, cell division, and membrane potential using the stain DiBAC4(5) in mutant strains of *E. coli* bacteria to help elucidate the compound's mechanism of action. Lastly, we determined if R327 is active against nongrowing cells. *In vitro* studies demonstrated that R327 was rapidly bactericidal to wild type *E. coli* reducing the population of viable cells below the threshold of detectability within 30 minutes. Washout experiments performed showed the effect of R327 was apparently irreversible. In luciferase experiments, it was demonstrated that R327 dramatically inhibited luciferase activity, indicating that it markedly compromised cellular energetics. Treatment with R327 led to the disassembly of the bacterial cell division protein complex which requires intracellular energy to remain assembled. R327 was observed to disrupt the membrane potential in *E. coli* imp mutant cells based on the entry of DiBAC4(5) into the cytoplasm resulting in fluorescence of cells. Finally, we found that R327 led to a rapid decrease in viable stationary phase cells that were not actively growing. These studies strongly indicate that R327 is rapidly and irreversibly bactericidal to both active and slow-growing cells and that itacts by disrupting cellular bioenergetics, potentially by disrupting the membrane potential and/or ATP synthesis.