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Soziema Dauda
soziema.dauda@pop.belmont.edu

Characterization of NBTI-induced single- and double-stranded DNA breaks mediated by *Neisseria gonorrhoeae* type II topoisomerases

Soziema Dauda[1]; Jessica Collins[2]; Rebecca Adams, Ph.D[1]; Neil Osheroff, Ph.D.[2]
[1]Belmont University, Nashville, TN, 37212; [2]Department of Biochemistry, Vanderbilt University, Nashville, TN, 37205

Neisseria gonorrhoeae has been ranked as one of five "urgent threats" for resistance by the Centers for Disease Control and Prevention. Previously, fluoroquinolones, which target the bacterial type II topoisomerases, gyrase and topoisomerase IV, were frontline treatments for gonorrhea. However, clinical use of this class has been discontinued due to the prevalence of resistance mutations in the genes encoding these enzymes. Consequently, a new class of gyrase/topoisomerase IV-targeted agents known as Novel Bacterial Topoisomerase Inhibitors (NBTIs) are being developed to combat this bacterial threat. Type II topoisomerases relieve torsional stress generated in the DNA double helix during replication, transcription, and recombination by introducing transient double-stranded breaks in DNA. Fluoroquinolones and NBTIs stabilize these DNA breaks, thus increasing levels of DNA scission. As such, we hypothesized that the novel NBTI, OSUAB-185, would increase levels of DNA cleavage mediated by *N. gonorrhoeae* type II topoisomerases. Using *in vitro* DNA cleavage assays, we determined that OSUAB-185 increases levels of single-stranded DNA cleavage mediated by *N. gonorrhoeae* gyrase and topoisomerase IV. Whereas double-stranded breaks mediated by *N. gonorrhoeae* gyrase were suppressed, matching previously reported topoisosomerase-NBTI interactions, double-stranded breaks mediated by topoisomerase IV were enhanced. Given its unique ability to increase levels of double stranded breaks, we focused our studies on the activity of OSUAB-185 against topoisomerase IV. Results indicate that at 10 μM OSUAB-185, the DNA cleavage/ligation equilibrium is reached by 10 minutes. Additional experiments confirm that NBTI-induced DNA cleavage enhancement is mediated by *N. gonorrhoeae* topoisomerase IV. Thus, this NBTI shows promise as a new antibacterial to effectively target *N. gonorrhoeae*. Future studies will monitor the activity of OSUAB-185 against fluoroquinolone- and NBTI-resistant *N. gonorrhoeae* type II enzymes, which supports our long-term goal of designing new drugs to overcome fluoroquinolone resistant infections.