Genes Targeted Peptide Nucleic Acids Kill *Mycobacterium Smegmatis*

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Abstract

Antisense peptide nucleic acids (PNAs) targeted to essential genes have been shown to be novel therapeutic compounds to inhibit bacterial growth. The PNAs are very stable, short synthetic nucleic acid analogues (10-12 bp) in which the sugar-phosphate backbone of natural nucleic acid has been replaced by a synthetic peptide backbone usually formed from N-(2-aminoethyl) glycine units. PNAs are very long-lived antisense constructs that inhibit expression of targeted genes at either the transcriptional or translational level. An investigation was undertaken to evaluate the ability of several antisense PNAs to inhibit extracellular *M. smegmatis* in Middlebrook 7H9 broth culture and intracellular *M. smegmatis* in J774A.1 murine macrophage cell line. Six PNAs obtained from a commercial supplier were designed to inhibit the expression of the following essential genes: *inhA* (a fatty acid elongase), *rpsL* (ribosomal S12 protein), *gyrA* (DNA gyrase), *pncA* (pyrazinamidase), *polA* (DNA polymerase I) and *rpoC* (RNA polymerase β subunit) of *M. smegmatis*. Each PNA was used at 20μM, 10μM, 5μM and 2.5μM concentrations to test whether they induced a dose dependent inhibition of *M. smegmatis* cultured in Middlebrook 7H9 broth at 37 °C. PNAs targeting *inhA* and *rpsL* exhibited strong growth inhibition at all four concentrations, whereas only 20μM concentration of PNAs targeting *pncA*, *polA* and *rpoC* genes strongly inhibited the growth of *M. smegmatis*. PNAs targeting *gryA* and a mismatch PNA targeting *dnaG* (DNA primase) did not inhibit *M. smegmatis* in pure culture. Six PNAs at 20μM, 2μM and 0.2μM concentrations targeting *inhA, rpsL, gyrA, pncA polA* and *rpoC* were also tested in J774A.1 murine macrophages infected with *M. smegmatis*. All six PNAs exhibited statistically significant growth inhibition *($p<0.05$)* of *M. smegmatis* in murine macrophages. Data from this study suggests that PNAs could be a novel therapeutic approach against *Mycobacterium* infections.

Key words: *M. smegmatis*, Peptide nucleic acid, murine macrophage, antisense therapy