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External Guide Sequences (EGS) Elicit Cleavage of Hepatitis B Virus RNA by RNase P

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RNAse P is an endogenous ribozyme system present in all cells which processes tRNA. Heterologous RNAs can be targeted for cleavage by RNAse P by the use of an external guide sequence (EGS). Hybridization between a "human EGS," resembling three-quarters of a tRNA molecule, and an appropriate site in the target RNA results in formation of double stranded aminoacyl acceptor and D stems. This tRNA precursor-like structure is cleaved by RNAse P in the target RNA, as if it were the 5′ leader sequence. Four EGSs were designed against a target RNA corresponding to the first 837 nucleotides of hepatitis B virus (strain adw), which includes parts of the pre-S2 and S coding region of the surface antigen (HBsAg). All EGSs elicited varying degrees of cleavage of the HBV RNA by human RNAse P or the catalytic RNA "M1" of *E.coli* RNAse P. Since resistance to nucleases is an important aspect of a viable ribozyme therapeutic, these three-quartered EGSs were transcribed using phosphorothioate ribonucleotides. Such modified EGSs were several orders of magnitude more resistant to nucleases than their entirely RNA counterparts in a HeLa cell S100. Importantly, some of these EGSs were able to elicit cleavage of the target RNA by M1 RNA and human RNAse P. We are in the process of testing the efficacy of these EGSs in HBsAg-expressing cells in culture.

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Synthesis and Biological Evaluation of D-(2S) and L-(2R)-1,3-Oxathiolanyl- and D-(2R)- and L-(2S)-1,3-Dioxolanyl-Nucleosides as Anti-HIV and Anti-HBV Agents

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Since racemic oxathiolane-C and dioxolane-T have been reported as potent anti-HIV agents, these unusual classes of nucleosides, whose 3'-CH_2 group was replaced by sulfur and oxygen, respectively, have attracted much attention as new anti-HIV agents. The enantiomers of oxathiolane-C and dioxolane-T have been synthesized by general methods developed by our laboratories and the comprehensive structure-activity relationships have been studied. It was found that the unnatural isomer, (-)-β-L-(2R,5S)-1,3-oxathiolanyl-cytosine was more potent and less toxic than the natural isomer, (+)-β-D-(2S,5R)-1,3-oxathiolanyl-cytosine or racemic mixture against HIV and HBV. However, (-)-β-D-(2R,4R)-dioxolanyl-thymine exhibited slightly more potent anti-HIV activity than (+)-β-L-(2S,4S)-dioxolanyl-thymine, while (-)-β-L-(2S,4S)-dioxolanyl-cytosine was more potent than (+)-β-D-(2R,4R)-dioxolanyl-cytosine against HIV and HBV. In order to determine if these findings are common in these classes of nucleosides, a series of D-(2S)- and L-(2R)-1,3-oxathiolanyl- and D-(2R)- and L-(2S)-1,3-dioxolanyl-nucleosides were synthesized and evaluated as anti-HIV and anti-HBV agents. However, the results that L-isomers are more active than D-isomers were only applied to the cytosine derivatives of both classes. From the structure-activity relationship studies, (-)-β-D-(2R,4R)-dioxolanyl-guanine was found to be a promising anti-HIV agent and, interestingly, some α-isomers also exhibited good anti-HIV activity. Synthesis, anti-HIV and anti-HBV activities of these classes of nucleosides will be presented (supported by NIH grants AI 25899, AI 32351, AI 33655, CA 52020 and Veterans Administration).