INGIBITION OF GENE EXPRESSION WITH ALTRITOL- AND HEXITOL-MODIFIED siRNAs

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The altritol nucleic acid (ANA) and hexitol nucleic acid (HNA) provides RNA-like nucleotides that are well suited for siRNA applications. So, altritol-modified siRNA targeting the *MDR1* gene can exhibit improved efficacy as compared to unmodified siRNAs

INTRODUCTION

There has recently been substantial interest in potential therapeutic exploitation of gene regulation based on RNA interference (RNAi). The endogenous regulatory mechanisms involved in the physiological role of RNAi can be activated via the delivery of short double stranded RNAs (siRNA) to the cell interior.

A variety of strategies for siRNA modification have been pursued including alterations in the backbone chemistry, 2'-sugar modifications, nucleobase modifications and others. These efforts have, in some cases, resulted in significant improvements in the biological effectiveness of siRNA and have provided important insights into how chemical modification affects function. In terms of overall design, understanding of the biochemical mechanism of RNA interference has provided important guidelines; first, the siRNA must maintain an A-form (RNA-like) duplex, second, the 5'-position on the antisense strand must be able to be phosphorylated, third, to be effective siRNAs should have low thermodynamic stability in the 5' antisense region. These general design principles can then be further evolved through the use of selected chemical modifications.

RESULTS AND DISCUSSION

Altritol-modified nucleic acids (ANAs) support RNA-like A-form structures when included in oligonucleotide duplexes. Thus altritol residues seem suitable as candidates for the chemical modification of siRNAs. Here we report that ANA-modified siRNAs targeting the *MDR1* gene can exhibit improved efficacy as compared to unmodified controls. This was particularly true of ANA modifications at or near the 3' end of the sense or antisense strands, while modification at the 5' end of the antisense strand resulted in complete loss of activity. Multiple ANA modifications within the sense strand were also well tolerated. Duplexes with ANA modifications at appropriate positions in

both strands were generally more effective than duplexes with one modified and one unmodified strand. Initial evidence suggests that the loss of activity associated with ANA modification of the 5'-antisense strand may be due to reduced phosphorylation at this site by cellular kinases. Treatment of drug resistant cells with MDR1-targeted siRNAs resulted in reduction of P-glycoprotein (Pgp) expression, parallel reduction in MDR1 message levels, increased accumulation of the Pgp substrate rhodamine 123, and reduced resistance to antitumor drugs. Interestingly, the duration of action of some of the ANA-modified siRNAs was substantially greater than that of unmodified controls. These observations suggest that altritol modifications may be helpful in developing siRNAs with enhanced pharmacological effectiveness.

Fig.1. The chemistry and structure of altritol-modified oligonucleotides. (A) The chemical structure of ANA oligonucleotides. (B) Model of an ANA-RNA duplex; the green strand represents the ANA-modified oligonucleotide.

REFERENCES

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