

Phosphorothioate oligonucleotides inhibit the intrinsic tenase complex.

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Abstract

Systemic administration of ISIS 2302, a 20-mer antisense phosphorothioate oligonucleotide targeting human intercellular adhesion molecule-1 mRNA, causes prolongation of plasma clotting times in both monkey and human studies. The anticoagulant effects of ISIS 2302 were investigated with both in vitro coagulation assays in human plasma and purified enzyme systems. At high oligonucleotide plasma concentrations (>100 microgram/mL), prolongation of the prothrombin and thrombin times was observed. In a thrombin time assay using purified components, high concentrations of ISIS 2302 inhibited thrombin clotting activity both by stimulating inhibition by heparin cofactor II and directly competing with fibrinogen for binding to anion binding exosite I. In contrast, low concentrations of ISIS 2302 (<100 microgram/mL) showed a selective, linear prolongation of the activated partial thromboplastin time (PTT). The rate limiting effect of 50 microgram/mL ISIS 2302, which prolonged the PTT to 1.5 times control, was identified by sequential modification of the clotting assay. Delaying addition of oligonucleotide until after contact activation failed to correct prolongation of the PTT. The calcium-dependent steps of the intrinsic pathway were individually assessed by adding sufficient activated coagulation factor to correct the PTT in plasma deficient in that specific factor. Addition of factor XIa, IXa, VIIIa, or Va failed to correct the PTT in the presence of ISIS 2302. In contrast, 0.2 nmol/L factor Xa corrected prolongation of the PTT in factor X-deficient plasma with or without oligonucleotide present. ISIS 2302 (50 microgram/mL) did not prolong a modified Russel viper venom time, suggesting no significant inhibition of prothrombinase. Thus, 50 microgram/mL ISIS 2302 prolonged the PTT by selectively inhibiting intrinsic tenase activity. ISIS 2302 showed partial inhibition of intrinsic tenase activity (to approximately 35% of control) at clinically relevant oligonucleotide concentrations in a chromogenic assay. This activity was oligonucleotide sequence-independent but required the phosphorothioate backbone, suggesting that inhibition of intrinsic tenase is a general property of this class of oligonucleotides. These results are relevant to both the therapeutic use of phosphorothioate oligonucleotides and the potential design of inhibitors of the intrinsic tenase complex, a novel target for anticoagulation. Copyright 1998 by The American Society of Hematology. PMID #9716589