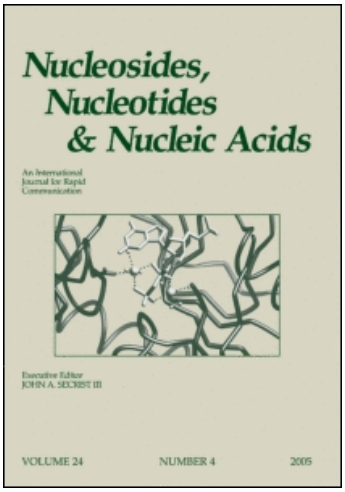

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Effects of Intravenous Infusion of Phosphorothioate Oligonucleotides on Coagulation, Complement Activation and Hemodynamics

Scott P. Henry[a]; David Monteith[a]; Doug J. Kornbrust[a]; Arthur A. Levin[a]
[a] Isis Pharmaceuticals, Carlsbad, CA

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EFFECTS OF INTRAVENOUS INFUSION OF PHOSPHOROTHIOATE OLIGONUCLEOTIDES ON COAGULATION, COMPLEMENT ACTIVATION AND HEMODYNAMICS

Scott P. Henry, David Monteith, Doug J. Kornbrust and Arthur A. Levin
Isis Pharmaceuticals, Carlsbad, CA 92008

ABSTRACT Several acute toxicities in monkeys have been associated with administration of phosphorothioate oligonucleotides including effects on coagulation and complement cascades. These effects are transient in nature and the severity is closely associated with peak plasma concentrations of the oligonucleotide. Similar properties have been observed for several oligonucleotides with different base sequences.

INTRODUCTION Phosphorothioate oligonucleotides (P=S oligos) are being developed as antisense therapeutics and are in clinical evaluation for as many a nine disease indications.[1] Several acute toxicities have been associated with administration of these compounds, including inhibition of coagulation and complement activation.[2] These effects have been shown to be transient in nature and closely associated with plasma concentrations of P=S oligo. The severity of the acute toxicities is correlated with the peak plasma concentrations, and therefore, can be controlled by limiting the extent of plasma exposure. Acute changes in coagulation and complement parameters have been observed with several oligonucleotides of different sequence. Therefore, these toxicities appear to be due to the chemical class and independent of base sequence. In this study we also demonstrate the acute toxicities are very predictable based on the extent of plasma exposure. Understanding this pharmacodynamic relationship will allow for the design of dose regimens that will avoid acute toxicities.

METHODS Data were compiled from a number of studies in which monkeys received intravenous infusion of P=S oligos (ISIS 2302, ISIS 3521/CGP 64128, or ISIS 5132/ CGP 69846). Doses of oligonucleotide ranged from 3 to 20 mg/kg, and infusion duration ranged from bolus injection to 2 hour infusion. Plasma samples were obtained at numerous time points up to 3 hours following infusion, and were analyzed for effects on clotting times (APTT and PT), complement activation (Bb, C3a and C5a split products), and plasma P=S oligo concentration. In addition, monkeys were monitored for acute changes in hematology or hemodynamic (central arterial blood pressure) parameters.

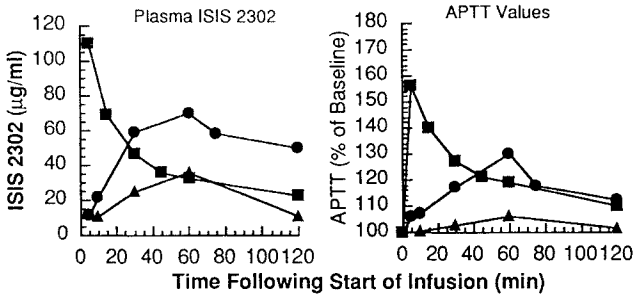

Figure 1. Plasma ISIS 2302 concentrations and APTT values immediately following 10 mg/kg IV bolus (square), 10 mg/kg over 60 minute infusion (circle) or 3.3 mg/kg over 60 minute infusion (triangle).

RESULTS AND DISCUSSION Intravenous administration of ISIS 2302 resulted in transient prolongation of coagulation which was manifested as an increase in clotting times. Following intravenous infusion of ISIS 2302, the intrinsic pathway appeared to be more sensitive to inhibition than to extrinsic pathway based on greater increases in activated partial thromboplastin times (APTT) as compared to prothrombin times (PT). Examination of the oligonucleotide exposure in plasma revealed a very close correlation between ISIS 2302 concentrations and increases in APTT, with clotting times increasing during infusion and returning toward baseline as oligonucleotide was cleared from plasma (Fig. 1). Maximal prolongation of clotting times corresponded to plasma Cmax and were generally within baseline variability by 2 to 4 hours following infusion. The clinical significance of the effects on coagulation are minor since the effects are transient, extent of increase in APTT is less than 2-fold over baseline, and only the intrinsic pathway was notably affected leaving other redundant pathways to maintain hemostasis. Plotting the plasma concentrations against the APTT effects reveals a linear relationship with ISIS 2302 exposure, and similar effects have been documented for other oligonucleotides.

Intravenous infusion of phosphorothioate oligonucleotides also resulted in activation of the alternative pathway of complement. Complement activation following 10 minute intravenous infusion of 5 mg/kg ISIS 3521/CGP 64128 resulted in increased levels of several complement split products including Bb (specific for alternative pathway) as well as C3a and C5a (anaphylatoxins) (Fig. 2). Like effects on the coagulation cascade, complement activation was temporally related to plasma oligonucleotide exposure (Fig. 2). The extent of split products generated, especially the anaphylatoxins, was proportional to peak plasma oligonucleotide concentrations.

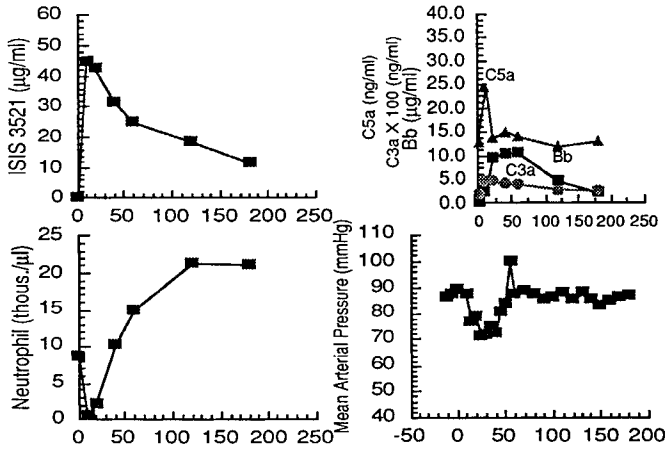

Figure 2. Correlation between plasma oligonucleotide concentration, complement activation, and secondary effects on neutrophil counts and blood pressure.

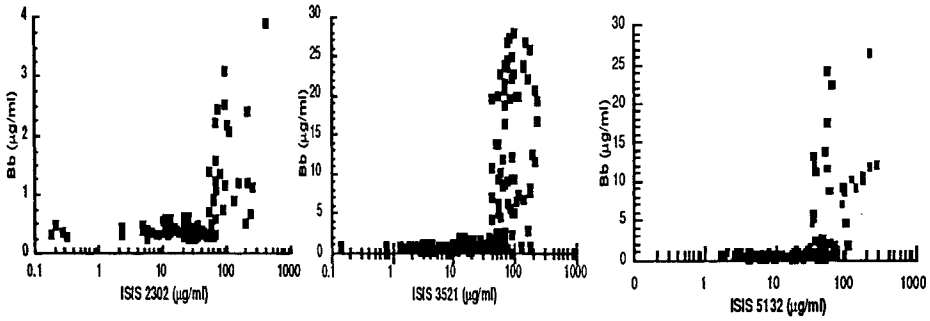

Figure 3. Threshold values for three different phosphorothioate oligonucleotides (ISIS 2302, ISIS 3521/CGP 64128, and ISIS 5132/CGP 69846.

C5a is a potent biological mediator, which produces secondary changes in hematologic or hemodynamic parameters (Fig. 2). These effects occur with close temporal relationship to plasma exposure and complement activation. Production of C5a has been shown to cause neutrophil activation and chemotaxis, which is reflected in transient neutropenia followed by neutrophilia[3]. C5a also is known to affect vascular tone and permeability, and is thought to be related to decreases in blood pressure occasionally associated with intravenous infusion of high doses of phosphorothioate oligonucleotides (Fig. 2).

Complement activation has been shown to be a common property of all phosphorothioate oligodeoxynucleotides examined to date. Correlating plasma

concentrations of oligonucleotide with complement split product concentrations (i.e., Bb), revealed a threshold concentration required for complement activation (Fig. 3). When plasma concentrations remain below this threshold the is no complement activation, however, complement activation is consistently observed when P=S oligo concentrations rise above this threshold. The threshold concentrations (45 to 50 μg/ml) for complement activation are qualitatively and quantitatively similar between three different phosphorothioate oligodeoxynucleotides examined (Fig. 3).

Data presented above indicate that acute toxicities related to P=S oligo administration are dose-dependent, related to plasma oligonucleotide concentrations, transient, and independent of nucleotide sequence. These toxicities occur at plasma P=S oligo concentrations that are greater than those experienced in the clinic. The plasma kinetics of P=S oligos are predictable between species and for different compounds, allowing for the design of safe dose regimens which will avoid exposure associated with acute toxicities.

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