Phase I Safety and Pharmacokinetic Profile of an Intercellular Adhesion Molecule-1 Antisense Oligodeoxynucleotide (ISIS 2302)

JOSEPHINE M. GLOVER, JANET M. LEEDS, TIMOTHY G. K. MANT, DIPTI AMIN, DANIEL L. KISNER, JOAN E. ZUCKERMAN, RICHARD S. GEARY, ARTHUR A. LEVIN and WILLIAM R. SHANAHAN, JR.


Accepted for publication May 12, 1997

ABSTRACT

Healthy male volunteers received single or multiple intravenous infusions of an intercellular adhesion molecule-1 antisense phosphorothioate oligodeoxynucleotide, ISIS 2302, in a rising-dose (0.06–2.00 mg/kg infused over 2 hr), double-blind, placebo-controlled trial. Brief, dose-related increases in activated partial thromboplastin time were seen at the time of peak plasma concentration ($C_{\text{max}}$). Clinically insignificant increases in C3a were seen after higher, repeated doses, but C5a, blood pressure and pulse were unaffected. No adverse events or other laboratory abnormalities were related to treatment with the drug. ISIS 2302 $C_{\text{max}}$ was linearly related to dose and occurred at the end of infusion. Plasma half-life for intact drug (53–54 min) and total oligonucleotide (67–74 min) were similar at the two doses (0.5 and 2.0 mg/kg) at which extensive pharmacokinetic data were collected. Nonlinear changes in area under the plasma concentration/time curve and steady-state volume of distribution with increasing dose suggested a saturable component to disposition. Metabolites co-migrating with n-1, n-2 and n-3 chain-shortened versions of ISIS 2302 appeared very rapidly in plasma, and disposition and metabolism appeared unaltered by repeated dosing. ISIS 2302 was well tolerated and behaved reproducibly with respect to plasma pharmacokinetics and expected side effects.

Multiple cell surface molecules responsible for activation and suppression of the immune system have been described and characterized (Springer, 1990). One such molecule, ICAM-1, is a transmembrane glycoprotein constitutively expressed at low levels on vascular endothelial cells and on a subset of leukocytes (Dustin et al., 1986; Rothlein et al., 1986; Simmons et al., 1988). In response to pro-inflammatory mediators, many cell types will up-regulate expression of ICAM-1 on their surface. ICAM-1 binds to the $\beta_2$ integrins, LFA-1 and Mac-1, expressed on leukocytes (Marlin and Springer, 1987; Diamond et al., 1990) and serves multiple functions in the propagation of an inflammatory process, the best characterized being facilitation of leukocyte emigration in response to inflammatory stimuli (Butcher, 1991; Furie et al., 1991; Oppenheimer-Marks et al., 1991).

Numerous studies have demonstrated an increase in ICAM-1 expression within tissues obtained from patients suffering from diseases with an inflammatory component. Although it is unlikely that increased ICAM-1 expression is the causative event for these diseases, continued expression of ICAM-1 probably contributes to the pathophysiology. ICAM-1 monoclonal antibodies have been used to demonstrate beneficial effects in a variety of animal models of disease, including pulmonary inflammation and asthma (Barton et al., 1989; Wegner et al., 1990), prevention of allograft rejection (Cosimi et al., 1990; Isobe et al., 1992), nephritis (Harning et al., 1992; Kawasaki et al., 1993), ischemic injury (Ma et al., 1992; Kelly et al., 1994), arthritis (Iigo et al., 1991) and contact dermatitis (Scheynius et al., 1993), which lend further support to the hypothesis that inhibitors of ICAM-1 function or expression could have broad therapeutic benefit.

ISIS 2302 is a phosphorothioate oligodeoxynucleotide 20 bases in length, designed to hybridize to the 3'-untranslated region of human ICAM-1 mRNA. ISIS 2302 selectively inhibits cytokine-induced ICAM-1 expression on a wide variety of human cells in vitro (Bennett et al., 1994; Mielo et al., 1994; Nestle et al., 1994). A murine equivalent, ISIS 3082, has been shown to be active in multiple murine pharmacology models of inflammation including prolongation of cardiac allograft survival (Stepkowski et al., 1994), dextran sulfate-induced...
colitis (Bennett et al., 1997) and endotoxin-induced pneumonia (Kumasaka et al., 1996). In each study, control oligonucleotides failed to demonstrate pharmacological activity, which suggests that the anti-inflammatory activity of ISIS 3082 was caused by inhibition of ICAM-1 expression. Down-regulation of ICAM-1 protein or message was demonstrated in involved tissue in the colitis and pneumonitis models.

The purpose of this first clinical trial with ISIS 2302 was to assess the safety and pharmacokinetics of intravenous administration of an anti-ICAM-1 oligodeoxynucleotide in healthy subjects before commencing pilot therapeutic trials in target disease states.

Methods

Study drug. ISIS 2302, a 20-base phosphorothioate oligodeoxynucleotide, was supplied by Isis Pharmaceuticals, Inc. as a sterile solution of 2.5 mg/ml in phosphate-buffered saline (pH 7.6). The percentage (area-percent) of full-length oligonucleotide, determined by capillary gel electrophoresis, was 92.25%, with the major impurity consisting of 4.95% n-1 deletion sequences. The product was 93.2% fully thioated, with the occurrence of more than one nontioated (phosphodiester) linkage in a single molecule being rare. ISIS 2302 is a racemic mixture, with an opportunity for chirality at each of its 19 phosphorothioate linkages.

Trial drug (or placebo) was administered by intravenous infusion in a volume of 80 ml over 2 hr in all cases. ISIS 2302 was diluted, as necessary, in sterile normal saline by a pharmacist at the trial unit. Sterile normal saline was used as placebo.

Subjects and sampling schedule. This was a double-blind, placebo-controlled study, conducted at Guy's Drug Research Unit Ltd. with local ethics committee approval. Four healthy male volunteers were recruited to each of seven single-dose groups (dose levels 0.06, 0.12, 0.24, 0.5, 1.0, 1.5 and 2.0 mg/kg ISIS 2302) and each of four multiple-dose groups (dose levels 0.2, 0.5, 1.0 and 2.0 mg/kg ISIS 2302) after screening medical history, examination and laboratory tests had shown no clinically significant abnormalities. One subject in each dose group was allocated (by use of a randomization list) to receive placebo while the others received active drug solution. Single-dose groups were dosed on day 1 only and multiple-dose groups were dosed on days 1, 3, 5 and 7. Groups were studied in a rising-dose fashion and multiple dosing commenced after the first five single-dose groups had completed the trial.

Subjects remained recumbent, with continuous electrocardiogram monitoring for 4 hr after the beginning of each infusion. The following were measured before and at intervals after each infusion: supine blood pressure and pulse, clotting screen (APTT, TT, PT), serum complement split products (C3a, C5a), neutrophil count (single-dose groups only), urine microproteins (retinol binding protein, N-acetyl-glucosaminidase, microalbumin; single-dose groups only) and standard laboratory safety screen (hematology, blood biochemistry, urinalysis). Serum samples were collected from multiple-dose groups at 14 and 21 days after the last infusion to be analyzed for the presence of antibodies to ISIS 2302.

Blood samples were taken for plasma ISIS 2302 concentration before and up to 24 hr after the beginning of infusion from all dose groups. More complete pharmacokinetic profiling was performed and urine collections were made up to 12 hr after the beginning of infusion in 0.5 and 2.0 mg/kg single- and multiple-dose groups.

Safety analyses. Laboratory safety screens, neutrophil counts, clotting screens and urine microproteins were analyzed according to standard, validated, laboratory procedures. Complement split products were measured by the Children's Nationwide Kidney Research Laboratory, Guy's Hospital, London, UK, with use of commercially available C3a and C5a des Arg 125I assay kits (Amersham Life Science, Arlington Heights, IL).

Procedure for detection of antibodies to ISIS 2302. This procedure was performed by Isis Pharmaceuticals Inc, Carlsbad, CA, with a modification of a previously described methodology (Lacy and Voss, 1989). Dispos able enzyme-linked immunosorbent assay plates (Corning Costar, Oneonta, NY) were coated with ISIS 2302. Uncoated areas on plates were blocked by incubating with 2% bovine serum albumin solution (Sigma Chemical, St Louis, MO) or 2% non-fat-dried Carnation milk powder. Aliquots of the diluted plasma samples (1:10 or 1:100) or equivalent volumes of diluted medium from a hybridoma cell culture line producing monoclonal antibodies which recognize ISIS 2302 (positive control) were added to the plates in triplicate, then incubated. Because isolated ISIS 2302 (and other phosphorothioate oligonucleotides) do not appear to be antigenic, these monoclonal antibodies to ISIS 2302 were raised by immunizing mice with ISIS 2302 conjugated to keyhole limpet hemocyanin. Plates were washed and then incubated with goat anti-human IgG or IgM conjugated with alkaline phosphatase (Sigma Chemical, St Louis, MO). After four further washes, plates were incubated with the alkaline phosphatase substrate, p-nitrophenylphosphate (Sigma Chemical, St Louis, MO) and the absorption at 405 nm was determined spectrophotometrically with a Titertek Multiskan MCC/340 plate reader (Lab Systems, Helsinki, Finland). Wells incubated with only a blocking agent (2% bovine serum albumin solution or 2% non-fat-dried milk) served as negative controls for these samples.

Results

Forty-four Caucasian, male subjects entered the trial, ranging in age from 20 to 35 years. One subject failed to
return for follow-up 7 days after dosing, but all other subjects completed the trial procedures.

**Safety.** A consistent and dose-related increase in APTT, whose magnitude varied between individuals but was of potential clinical importance in the highest dose groups, was seen in subjects who received single or multiple doses of 0.5 mg/kg ISIS 2302 and above (fig. 1). The maximum increase in APTT was seen between 1 and 2 hr after the beginning of infusion, and values returned to base line (or below) within 2 to 4 hr after the end of infusion. The greatest increases in APTT were seen in the 2.0 mg/kg single- and multiple-dose groups. End of infusion (hour 2) APTTs ranged from 49.4 to 54.3 sec in the three 2.0 mg/kg single-dose subjects, and from 39.9 to 51.5 sec on days 1, 3, 5 and 7 in the three 2.0 mg/kg multiple-dose subjects (normal range, 27.0–36.2 sec). Median APTT values were 45.4, 46.9, 46.4 and 48.0 sec on days 1, 3, 5 and 7, respectively, in the multiple-dose 2.0 mg/kg subjects.

Comparison of results from single- and multiple-dose groups showed no consistent tendency for exaggeration or attenuation of this effect after multiple dosing: the highest median APTT values were seen on day 1 (first infusion) in the 0.5 and the 1.0 mg/kg dose groups, and on day 7 (fourth infusion) in the 2.0 mg/kg dose group. By combining the hour 2 APTT values for all four infusion days, a linear relationship of APTT to dose over the investigated multiple-dose range of 0.2 to 2.0 mg/kg can be appreciated (fig. 2).

Much smaller, clinically insignificant and less clearly dose-related increases in PT and TT occurred in subjects who received ISIS 2302. TT rose slightly above the reference range in some cases but PT did not. The maximum median increases in these parameters (approximately 6% for PT and 10% for TT) occurred between 30 and 120 min after the beginning of a single infusion, and recovery occurred within 2 hr from this time. Bleeding time was unaffected by the trial drug.

Complement C5a split product concentrations remained unchanged throughout the study. Single-dose groups showed no difference in complement C3a concentrations between subjects who had received active drug and placebo. Among multiple-dose group subjects, a small increase in median C3a concentration was seen after the fourth dose of 1.0 mg/kg ISIS 2302 and the third dose of 2.0 mg/kg. A larger increase was seen after the fourth dose for 2.0 mg/kg (fig. 3). The magnitude of increase was not of clinical significance in any individual. The maximum change in this parameter (up to 130% median increase from base line) was seen at 60 or 120 min after the beginning of infusion. Substantial recovery had occurred by 4 hr, but sampling was not continued beyond this point.

Electrocardiogram, supine blood pressure and pulse were unaffected by dosing. No serious adverse events were reported nor was any particular adverse event seen occurring with disproportionate frequency after active drug with the exception of headache (seven ISIS 2302, one placebo; expected ratio 3:1), whose incidence was not related to dose of ISIS 2302. Review of the median percent change from base line in laboratory safety variables revealed no dose-related differences between active drug- and placebo-treated subjects, with the exceptions of the above-mentioned changes in APTT and C3a levels. Importantly, no consistent changes were seen in white cell indices (including neutrophil count) as a result of dosing with this ICAM-1 inhibitor.

Predose and day 22 plasma samples from each multiple-dose group subject were analyzed for IgG and IgM antibodies to ISIS 2302. No indication was seen of the presence of drug-specific antibodies in subjects treated with ISIS 2302 under the conditions of this trial.

**Pharmacokinetics.** Results are expressed as both the amount of parent compound and of total oligonucleotide present (defined as the sum of intact ISIS 2302 plus apparent n-1, n-2 and n-3 chain-shortened metabolites). In plasma, metabolites shorter than apparent 17-mer were not generally detected (fig. 4). Although substantial amounts of material co-migrated with shorter oligonucleotide standards in urine (fig. 5), these were not quantitated because base-line separation was not achieved reliably.

Plasma concentration/time curves for subjects who received single infusions of 0.5 and 2.0 mg/kg are shown in figure 6. Pharmacokinetic parameters were calculated for each subject in the 0.5 and 2.0 mg/kg single-dose groups (table 1) by use of an open, one-compartmental model, which yielded a good fit to the data by both statistical and visual criteria, although the plasma concentration/time curve appeared biphasic. Plasma half-life appeared consistent between doses. Although end-of-infusion plasma concentrations of ISIS 2302 increased linearly with increasing doses of drug (fig. 7), AUC values increased disproportionately; a 4-fold increase in single dose produced a 7-fold increase in AUC of both intact drug and total oligonucleotide. This suggestion of a saturable component to the disposition of ISIS 2302 is supported by the results of pharmacokinetic modeling, which demonstrated a reduction in volume of distribution and plasma clearance as dose increased (table 1), and by observations in animals with other oligonucleotides (Srinivasan and Iversen, 1995; Rifai et al., 1996).

During 2-hr, single infusions of ISIS 2302, metabolites co-migrating with synthesized n-1, n-2 and n-3 chain-shortened forms (shortmers) of the intact drug appeared very rapidly in plasma, constituting 20% of total oligonucleotide after 30 min of infusion. Interestingly, the relative proportion of total oligonucleotide constituted by full-length oligo and n-1, n-2 and n-3 shortmers remained relatively constant during the 2 hr of study drug infusion and for at least the 4 hr postinfusion during which metabolites were measured.

![Fig. 1. Median APTT (expressed as percent of control) after each of four doses of ISIS 2302 (n = 3) or placebo (n = 4). Placebo or ISIS 2302 (0.2, 0.5, 1.0 and 2.0 mg/kg) was administered by 2-hr intravenous infusion on days 1, 3, 5 and 7.](image-url)
Therefore, intact drug remained the majority of oligonucleotide present until levels fell below the limits of detection (fig. 8).

There was no evidence of accumulation of oligonucleotide in plasma with alternate day dosing by infusion over 2 hr: comparison of end-of-infusion concentrations of intact drug or total oligonucleotide after the first through the fourth multiple dosings with 0.2, 0.5, 1.0 or 2.0 mg/kg ISIS 2302 failed to reveal a consistent trend to change in these values (fig. 9). Furthermore, there were no clear differences in the proportions or concentrations of intact drug and metabolites over time between the first and fourth infusions, giving no suggestion that repeated dosing with ISIS 2302 caused induction or inhibition of its own metabolism.

Urine samples from the 1.0 and 2.0 mg/kg multiple-dose groups were analyzed for concentrations of intact drug and metabolites. Very low concentrations of ISIS 2302 and metabolites were present in urine. Intact drug and the n-1, n-2 and n-3 shortmers could be measured, and the quantity of shorter metabolites, although not well resolved, could be visually estimated from electropherograms. The amount of intact drug excreted over 6 hr after the beginning of infusion averaged approximately 0.05% of the administered dose, and the estimated total excretion of parent drug and metabolites in this period was less than 0.5% of the total dose.

Discussion

The first objective of this trial was to establish the safety of a range of single and multiple doses of ISIS 2302, a drug which had not previously been administered to humans. This objective was completed successfully, allowing the drug to progress into phase II trials in a variety of therapeutic indications.

In cynomolgus monkeys, ISIS 2302 has consistently caused anticoagulant effects (increased APTT) and alternative pathway complement activation at plasma concentrations in excess of 30 and 50 \(\mu\)g/ml, respectively, effects which are re-
related in magnitude to peak plasma drug concentration (Henry et al., 1997). In this trial, single- and multiple-dose groups demonstrated increases in APTT at doses of 0.5 mg/kg ISIS 2302 and above. These increases were very clearly dose-related in magnitude, just as peak plasma drug concentration was linearly related to dose, and did not appear to attenuate or accumulate with multiple dosing. The peak anticoagulant effect was seen at the time of peak plasma concentration of both intact ISIS 2302 and total oligonucleotide (at the end of infusion), and changes recovered rapidly and spontaneously within 2 to 4 hr after the end of infusion, the same period during which oligonucleotide was detectable in plasma after higher doses. The mechanism of this effect is not yet fully elucidated, but it is known that ISIS 2302, like other phosphorothioate oligonucleotides, is a large, polyanionic molecule which binds reversibly to several proteins, including thrombin (Henry et al., 1994). The reliable relationship between plasma drug concentration and anticoagulant effect will allow maximum doses/rates of infusion for future trials to be calculated on the basis of maximum acceptable increase in APTT.

No effect on the C5α complement split product was seen, but small, brief increases in C3a appeared after repeated infusions of higher doses of ISIS 2302. The mechanism of complement activation or conversion by ISIS 2302 observed in this study is also not known although this is also suspected to be related to protein binding (Henry et al., in press, 1997) or nonspecific enzymatic degradation by leukocyte proteases. Nonclinical experiments are underway to investigate these possibilities. Rapid fluctuations in neutrophil count and hemodynamic changes, thought to be related to complement activation, have been observed at higher dose levels and infusion rates with ISIS 2302 and other phosphorothioate oligonucleotides in monkeys (Galbraith et al., 1994; Henry et al., 1997). These variables were also measured at frequent intervals after the beginning of infusion in the single-dose subjects in this trial, but no significant changes nor any trends to change were observed. It is likely that any phosphorothioate oligonucleotide of sufficient chain length will cause similar effects on clotting and complement functions in animals and humans if administered in such a way as to exceed threshold peak plasma drug concentrations.

Clinically, ISIS 2302 was well tolerated. There were no clinical signs, symptoms or changes in routine laboratory safety parameters which were related to ISIS 2302. Specifically, there was no evidence of an effect of this ICAM-1 inhibitor on gross immune function or on the white blood cell indices measured. This is not surprising because ICAM-1 is only one of the many mediators involved in regulating overall immune function. Consistent with experience at Isis in other animal and clinical trials with similar oligonucleotides, there was no evidence of antibody formation to ISIS 2302.

These results are also important in that this is the first description of the use of CGE to measure nonradiolabeled, systemically administered, phosphorothioate oligonucleotide in humans. Other authors have described the human pharmacokinetics of intravenously injected 35S-labeled GEM 91 (a 25-mer) (Zhang et al., 1995), intradermal 14C-labeled afovirsen sodium (a 20-mer) (Crooke et al., 1994) and unlabeled OL/1)p53 (a 20-mer) (Bayever et al., 1993), all phosphorothioate oligodeoxyribonucleotides.

The results of pharmacokinetic analysis in this trial are generally consistent with those obtained with other systemically administered phosphorothioate oligonucleotides in animals (Cossum et al., 1993, 1994; Agrawal et al., 1995) and humans (Bayever et al., 1993, 1995; Agrawal et al., 1994, 1995; Zhang et al., 1995), although there are differences with respect to the importance of urinary excretion and the presence of a terminal half-life. The present study demonstrates a rapid distribution phase, corresponding to the distribution phase seen in animal studies, and the volume of distribution appears to be consistent with previous data. This study differs from previous studies in that the terminal half-life described in studies with radiolabeled drug was not observed. This difference may be related to differences in assay sensitivity and the ability to measure single-nucleotide metabolites by radiochemical analysis. However, compared with unmodified oligonucleotides with phosphodiester backbones, the plasma half-life of intact drug is long. An additional difference between reports lies in the assessment of the importance of urinary excretion. In this study and those conducted with 14C-labeled afovirsen sodium (Cossum et al., 1993, 1994; Crooke et al., 1994), urine was found to be a minor route of excretion of oligonucleotides (or of radioactivity derived from drug), whereas studies with 35S-labeled 20- and 25-mers showed substantial urinary excretion of drug.
TABLE 1
Mean plasma pharmacokinetic parameters derived for ISIS 2302 and total oligonucleotide from those groups (n = 3) which received a single 2-hr infusion of 0.5 or 2.0 mg/kg ISIS 2302

<table>
<thead>
<tr>
<th>Parameter*</th>
<th>0.5 mg/kg Dose Group</th>
<th>Total Oligonucleotide</th>
<th>2.0 mg/kg Dose Group</th>
<th>Total Oligonucleotide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vss (ml/kg)</td>
<td>ISIS 2302</td>
<td>155.4 ± 13.6</td>
<td>121.9 ± 5.6</td>
<td>97.5 ± 7.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-24&lt;/sub&gt; (µg/min/ml)</td>
<td>ISIS 2302</td>
<td>249.7 ± 43.8</td>
<td>392.7 ± 92.9</td>
<td>1824.6 ± 111.1</td>
</tr>
<tr>
<td>K&lt;sub&gt;1/2&lt;/sub&gt; (min)</td>
<td>ISIS 2302</td>
<td>54.4 ± 16.1</td>
<td>66.7 ± 18.2</td>
<td>52.9 ± 6.0</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (µg/ml)</td>
<td>ISIS 2302</td>
<td>1.60 ± 0.16</td>
<td>2.30 ± 0.26</td>
<td>10.3 ± 0.06</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;/dose (µg/ml)</td>
<td>ISIS 2302</td>
<td>3.21 ± 0.33</td>
<td>4.60 ± 0.52</td>
<td>5.17 ± 0.46&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Clearance (ml/min/kg)</td>
<td>ISIS 2302</td>
<td>2.07 ± 0.48</td>
<td>1.33 ± 0.34</td>
<td>1.28 ± 0.12&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*V<sub>ss</sub>, steady-state volume of distribution; AUC<sub>0-24</sub>, area under the plasma concentration/time curve extrapolated to infinity; K<sub>1/2</sub>, plasma half-life; C<sub>max</sub>, maximum plasma concentration.

<sup>a</sup>p < .05, as compared with the 0.5 mg/kg dose group.

Fig. 7. Plasma concentrations of ISIS 2302 determined at the end of single, 2-hr infusions of ISIS 2302.

derived radioactive material, mostly in the form of metabolites (Agrawal et al., 1995; Zhang et al., 1995). These differences could be accounted for by a difference in the route of excretion of carbon- and sulfur-related moieties resulting from metabolism of phosphorothioate oligodeoxynucleotides. Zhang et al. (1995) describe the urinary excretion of approximately 70% of GEM 91-derived 35S over 96 hr after a single human dose, whereas Cossum et al. (1993, 1994) and Crooke et al. (1994) found approximately 50% and 30% of afovirsen sodium-derived 14C in expired air and only approximately 15% and 10% in urine after single rat and human doses, respectively.

Bayever et al. (1993) used high-performance electrophoretic chromatography to measure cumulative urinary excretion of an unlabeled 20-mer phosphorothioate oligodeoxynucleotide (OL(1)p53) given by continuous intravenous infusion at a rate of 0.05 mg/kg/hr (1.2 mg/kg/day) for 10 days to five patients with acute myelogenous leukemia or myelodysplastic syndrome. Cumulative urinary excretion of apparently undifferentiated oligonucleotide ranged from 42 to 63% of administered drug. Plasma drug levels were not measured directly, but peak plasma concentrations (2.1–6.4 µg/ml) and half-life (4.9–14.7 days) were calculated from the rate of urinary excretion. There are considerable methodological differences between the high-performance electrophoretic chromatography technique used by Bayever et al. (1993) and the CGE technique (Leeds et al., 1996) used in the present study to measure drug concentrations, including methods of extraction, scale of analytic method and ability to identify intact drug and metabolites. Furthermore, plasma half-life was not directly measured by Bayever et al. (1993), but calculated from urinary excretion. Differences in plasma half-life and the relative importance of urinary excretion found in these two studies could be caused by differences in dosing regimen (continuous infusion for 10 days versus 2-hr infusions every other day) and/or quantitative methodology. Continuous infusion might saturate plasma binding capacity, cellular uptake and/or renal proximal tubular reabsorption.

No other accounts of human pharmacokinetic results after multiple dosing are available for comparison. In this trial, no accumulation of drug or metabolites was seen in plasma, nor was there any apparent change in the kinetics or metabolism of ISIS 2302 in plasma with repeated administration. Similar plasma pharmacokinetic behavior has been seen in monkeys ( Isis Pharmaceuticals; A. Levin, unpublished data). Although tissue levels cannot be determined in clinical trials, we can speculate, by extrapolation from animal data demonstrating tissue half-lives on the order of 24 to 120 hr, depending upon the tissue and the dosing regimen (Cossum et al., 1993; Isis Pharmaceuticals, A. Levin, unpublished data), that concentrations of ISIS 2302 and its metabolites in target tissues may be maintained with an alternate-day regimen.

This study showed that the peak plasma concentration and AUC for total oligonucleotide was approximately 50% higher than for ISIS 2302 alone. Nonclinical studies have indicated that synthesized n-1, n-2 and n-3 chain-shortened metabolites are capable of inhibiting ICAM-1 expression and of causing the toxicities typical of phosphorothioate oligonucleotides ( Isis Pharmaceuticals, F. Bennett and A. Levin, unpublished data).

The observation that the relative proportion of total oligonucleotide constituted by full-length oligo and n-1, n-2 and n-3 shortmers remained fairly constant throughout the 2-hr infusion period and for at least 4 hr postinfusion suggests that ISIS 2302 is shortened one base at a time by exonucleases that compete at a constant rate for substrate irrespective of chain length [at least ≥ n-3 mers (17-mers)] and base sequence. This constancy of proportionality (i.e., the very slow increase in the proportion of metabolites over time), present by 30 min into the infusion, the time at which the first pharmacokinetic samples were drawn, further suggests that metabolism in plasma is largely complete within 30 min. Potential explanations for this phenomenon include: 1) the buildup in plasma of a nuclease-inhibitory metabolite; 2) the presence of a significant impurity in the drug product with a different rate of metabolism; and 3) a differential rate of metabolism for the Rp and Sp diastereoisomers present at each phosphorothioate linkage. The generation of an inhibi-
tory metabolite seems implausible because the rate and pattern of metabolism appear constant through time and across a 4-fold dose range. With the exception of chirality, the ISIS 2302 administered was high in analytical purity (see "Methods"), and therefore the presence of a significant impurity seems an unlikely explanation. However, phosphorothioate diastereomeric selectivity has been reported for exonucleases (Burgers and Eckstein, 1979; Spitzer and Eckstein, 1988), and this offers a potential explanation for the pattern of metabolism observed in this study. If pharmacokinetic modeling is performed assuming exonuclease base deletion in sequence, a probability of 0.5 at each linkage for rapid (Sp) or slow (Rp) cleavage and a "half-life" of 3 hr for the Rp isomers and 3 min for the Sp isomers, a metabolic pattern very similar to that observed in this study is produced. The absence of significant urinary excretion and the failure to detect a buildup of metabolites, either long (17- to 19-mers) or short (<17-mers), suggests that ISIS 2302 is principally cleared from plasma by extravascular tissue distribution and subsequent cellular uptake.

The fact that the major plasma metabolites detected in this study co-migrated with standards shortened by one, two or three nucleotides from the 3' end of the ISIS 2302 molecule is also consistent with a hypothesis that phosphorothioate oligonucleotides undergo metabolism by exonucleases which remove single bases in a sequential manner from the end of the molecule. Alternatively, nuclease activity might remove pairs or triplets of bases from the end of the molecule to produce n-2 and n-3 metabolites. Although CGE cannot determine the sequence of the apparent 19-, 18- and 17-mers seen, the former hypothesis is more consistent with the observed metabolic profile and is inviting in that phosphodiester oligodeoxynucleotides in plasma undergo exonuclease digestion from their 3' end (Eder et al., 1991). We would further hypothesize that the shortmers resulting from such a process would eventually be catabolized in much the same way as endogenous nucleotides. This hypothesis is supported by the previous finding (described above) that approximately 50% of 14C radiolabel derived from a similar phosphorothioate oligonucleotide, labeled at the C-2 position of thymidine, was eliminated from rats as carbon dioxide in expired air (Cossum et al., 1993, 1994). Biliary excretion is unlikely to play any significant role because fecal elimination of labeled, intravenously administered phosphorothioates has been minimal in rodents (Cossum et al., 1993; Agrawal et al., 1995).

Overall, the predictability of the clinical profile and pharmacokinetics resulting from repeated infusions of ISIS 2302...
in this study gave the confidence necessary to allow pilot therapeutic trials in indications including renal transplantation, inflammatory bowel disease, rheumatoid arthritis and porosiasis to begin.

Acknowledgments

The authors thank C.Frank Bennett, Ph.D. for his scientific review of this paper.

References


Send reprint requests to: William R. Shanahan, Jr, Isis Pharmaceuticals Inc., 2292 Faraday Avenue, Carlsbad, CA 92006-7208.