Pharmacokinetics and Pharmacodynamics of an Antisense Phosphorothioate Oligonucleotide Targeting Fas mRNA in Mice

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ABSTRACT
ISIS 22023 is a modified phosphorothioate antisense oligonucleotide targeting murine Fas mRNA. Treatment of mice with ISIS 22023 reduced Fas expression in liver in a concentration-dependent and sequence-specific manner, which completely protected mice from fulminant death induced by agonistic Fas antibody. In this study, we characterized the relationships in mice between total dose administered, dose to the target organ, and ultimately, the intracellular concentration within target cell types to the pharmacologic activity of ISIS 22023. After subcutaneous injection, ISIS 22023 distributed to the liver rapidly and remained in the liver with the t1/2 ranging from 11 to 19 days, depending on dose. There were apparent differences in patterns of uptake and elimination in different types of liver cells. Oligonucleotide appeared within hepatocytes rapidly, whereas the peak concentrations in Kupffer cells were delayed until 2 days after dose administration. Hepatocytes cleared oligonucleotide the most rapidly, whereas Kupffer cells appeared to retain oligonucleotide longer. The reduction of Fas mRNA levels (pharmacodynamic response) paralleled the increase of oligonucleotide concentration in mouse liver with maximum mRNA reduction of 90% at 2 days after a single 50 mg/kg subcutaneous administration. Moreover, the pharmacodynamics of ISIS 22023 correlated better with the pharmacokinetics in hepatocytes, supporting the concept that the presence of oligonucleotide in target cells results in reductions in mRNA and, ultimately, pharmacologic activity. These results provide a comprehensive understanding of the kinetics of an antisense drug at the site of action and demonstrate that the reductions in mRNA induced by this antisense oligonucleotide correlate with its concentrations in cell targets.

A guiding principle in pharmacology is that there should be a correlation between dose and pharmacologic activity of a therapeutic agent. At times it is not always possible to define such relationships because of poor absorption, first-pass effects, elimination, or any one of a multitude of factors. In practice, plasma concentrations are often used as a surrogate for dose, thereby correcting for absorption, elimination, or metabolic factors. Clearly, dose administered and plasma levels are both surrogates for understanding the pharmacokinetics at the target site. Although these principles are well accepted and thoroughly validated for low molecular weight drugs, with antisense therapeutic agents there have always been questions about pharmacokinetics and tissue uptake. Little is known about the effective concentrations at the target sites for antisense oligonucleotides.

Phosphorothioate oligodeoxynucleotides have been studied extensively in recent years as potential antisense therapeutic agents and have been shown to be effective in the treatment of a broad range of diseases, including viral infections, inflammatory diseases, and various types of cancer (Monia et al., 1992; Bennett et al., 1996; Cowsert, 1997). The first antisense phosphorothioate oligodeoxynucleotide drug, Vitraseven, was approved by the FDA in 1998 for treatment of cytomegalovirus retinitis.

ISIS 22023 is a 20-mer phosphorothioate oligonucleotide that incorporates 2′-O-(2-methoxy) ethyl (MOE) modification on the five nucleotides on both 3′- and 5′-termini (Zhang et al., 2000). MOE modifications provide enhanced resistance to nucleases, provide a longer target organ half-life, and have reduced toxicity (McKay et al., 1999; Henry et al., 2000). In addition, these modifications also increase the affinity of an antisense oligonucleotide for complementary target mRNA, resulting in enhanced potency and specificity (Altmann et al., 1996; Baker et al., 1997; McKay et al., 1999). Although the pharmacokinetics, tissue, suborgan, and subcellular distribution of phosphorothioate oligonucleotides have been well characterized in rodents (Cossum et al., 1993; Agrawal et al., 1995; Zhang et al., 1995; Graham et al., 1998), primates (Grindel et al., 1998), and humans (Agrawal et al., 1991; Iversen et al., 1994; Glover et al., 1997; Stevenson et al., 1999), this is the first complete correlation of the pharmacokinetics at the target site. Further studies are required to fully characterize the pharmacokinetics and pharmacodynamics of ISIS 22023, particularly in nonhuman primates and humans.

ABBREVIATIONS: MOE, 2′-O-(2-methoxy) ethyl; CGE, capillary gel electrophoresis; AUC, area under the concentration-time curve.
kinetics and pharmacodynamics of an antisense oligonucleotide.

ISIS 22023 was designed to target a sequence within the translated region of the murine Fas mRNA. Previous studies have shown that ISIS 22023 inhibited Fas expression in a dose-dependent and sequence-specific manner both in vitro and in vivo (Zhang et al., 2000). Treatment with ISIS 22023 and its subsequent reduction in Fas mRNA levels have been shown to protect mice from liver injury in both agonistic Fas antibody and acetaminophen-induced fulminant hepatitis models and to completely protect mice from death induced by antagonistic Fas antibody.

For an antisense oligonucleotide to exhibit a pharmacologic response, the administered agent must distribute to its target organ and be taken up by the target cells, where it can bind to its cognate mRNA. In this study, we characterized the pharmacokinetics and pharmacodynamics of ISIS 22023 in mouse liver, the target organ, and in the different cell types within that organ. The results demonstrated that the time course of mRNA reduction (pharmacologic activity) correlated closely with the time course of ISIS 22023 concentrations in hepatocytes and that Fas mRNA reduction in mouse liver protected mice from Fas antibody-induced fulminant hepatitis.

Materials and Methods

Oligonucleotides. ISIS 22023 is a 20-nucleotide phosphorothioate antisense oligonucleotide with MOE modifications at positions 1 through 5 and 15 through 20 (underlined below). In addition, the cytosines in the 5′- and 3′-ends of the compound were modified to contain a 5-methyl group (5-methyl cytosine, Cm). The sequence of ISIS 22023 is TCGCmGmACmCmCmTTTGmTTTGmGmCG. ISIS 13866, a 2′-MOE modified oligonucleotide at positions 15 through 21 with a sequence of 5′-GCT mTT GCT CTT CTT CmTT GCG mTT TTT-3′, was used as the internal standard for quantitation of ISIS 22023 in plasma and tissues. ISIS 22023 and ISIS 13866 were synthesized using an automated DNA synthesizer model 380B (Applied Biosystems, Inc., Foster City, CA) and purified as previously described (Baker et al., 1997). The purity of the compounds used in this study was found to be 98.9% and 98.8% for ISIS 22023 and ISIS 13866, respectively.

Animals and Treatments. Female BALB/c mice, 8 weeks old (16–25 g), were purchased from The Jackson Laboratory (Bar Harbor, ME) and maintained in compliance with Isis Institutional Animal Care and Use Committee Guidelines in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited facility. The animals were housed in polycarbonate cages (three mice per cage) and allowed food and water ad libitum. ISIS 22023 solutions were formulated in sterile saline at concentrations of 1, 2, 3, and 5 mg/ml. The mice were randomly assigned to three groups such that three mice were sacrificed at each time point per group. Group 1 received a single subcutaneous (s.c.) injection of ISIS 22023 at a dose of 10, 20, 35, or 50 mg/kg (dose volume, 10 ml/kg). Group 2 was treated with a single s.c. injection of 50 mg/kg ISIS 22023 and was used to study suborgan kinetics. Group 3 was treated with one, two, and three doses of 50 mg/kg of ISIS 22023 administered subcutaneously every other week.

At each scheduled sampling time point (see Table 1) for groups 1 and 3, three mice were anesthetized using Avertin (containing 1.25% isobutyl alcohol, 1.25% tert-amyl alcohol, and 0.9% saline) and blood samples were collected from each mouse by cardiac puncture using EDTA as the anticoagulant. Approximately 100-μl aliquots of liver from each mouse were homogenized in 1 ml of RLT buffer (Qiagen, Santa Clarita, CA) containing 10 μl of β-mercaptoethanol and 0.35 μg oligonucleotide in plasma and liver, respectively.

**Sample Extraction and Analysis of ISIS 22023 in Plasma and Liver.** An aliquot of plasma (100 μl) for each sample was spiked with a known concentration of internal standard and extracted using solid-phase extraction and analyzed using CGE analysis (Leeds et al., 1996).

For liver samples, the method was similar except that tissues were weighed, homogenized in a Fastprep homogenizer (BIO 101, Inc., Vista, CA), and then the material was extracted as described (Leeds et al., 1996) with the exception that a phenyl-bonded SPE column (Supelco Inc., Bellefonte, PA) was used. Extraction of oligonucleotide from suborgan fractions was the same as previously described (Graham et al., 1998). Extracted samples were analyzed by CE using a Beckman P/ACE model 5010 capillary electrophoresis instrument (Beckman Instruments, Irvine, CA) with UV detection at 260 nm.

The concentrations of ISIS 22023 and metabolites in the plasma and liver samples were calculated from the ratio of the absorbance, based on the starting concentration of internal standard (ISIS 13866) as previously described (Leeds et al., 1996). The limit of quantitation for this assay has been estimated to be 0.07 μg/ml and 0.35 μg oligonucleotide in plasma and liver, respectively.

**RNA Isolation and RNaSe Protection Assay.** Total RNAs were extracted from mouse liver using the RNaseasy kit (Qiagen). An RNaSe protection assay was performed as suggested in the RiboQuant manual (PharMingen, San Diego, CA). RNaSe protection assay template mAp-3 and the custom templates (PharMingen) were used as probes. Total RNA (20 μg) of each treatment, either from cell culture or mouse liver, were used and analyzed on 6% denaturing polyacrylamide gel. Gels were then scanned by a PhosphorImager (model 300 Series, Molecular Dynamics, Sunnyvale, CA).

**Pharmacokinetic Analysis.** The plasma concentration-time profile after a single subcutaneous administration of 50 mg/kg ISIS 22023 was fitted to a one-compartment open model using WinNonlin 1.5 (Scientific Consulting, Inc., Cary, NC). ISIS 22023 was rapidly cleared from plasma and distributed into tissues. Whole body clear-
ance cannot be monitored using the terminal elimination phase in plasma because the existing methods lack sufficient sensitivity to assay such low levels of oligonucleotides. However, the elimination of ISIS 22023 from liver could be measured directly, and the liver concentration data after single subcutaneous injection of 10 to 50 mg/kg were analyzed using a noncompartmental model (WinNonlin 1.5). Cell-specific kinetics showed that the hepatocyte-concentration (parenchymal cells) data declined in a bi-exponential fashion over time. Moreover, noncompartmental analysis indicated that both liver uptake and elimination of ISIS 22023 might be saturated at high dose. Because of the limited number of observations collected, the model was simplified by choosing a two-compartment linear model (WinNonlin 1.5).

Pharmacodynamic Analysis. The relation between Fas mRNA reductions and ISIS 22023 concentrations in hepatocytes was characterized using a pharmacodynamic model. Fas mRNA levels following ISIS 22023 treatment were normalized with the Fas mRNA levels from control animals (treated with saline):

\[ E = \frac{mRNA_{\text{treated}}}{mRNA_{\text{control}}} \times 100 \]  

(1)

The relationship between inhibitory activity of Fas mRNA \( E \) and concentration of ISIS 22023 in liver was described by adopting an inhibitory sigmoidal \( E_{\text{max}} \) model:

\[ E = \frac{E_{\text{max}} - (E_{\text{max}} - E_0)C^n}{EC_{50}^n + C^n} \]  

(2)

where \( E_{\text{max}} \) is the maximum level of Fas mRNA, \( E_0 \) is the baseline level, \( EC_{50} \) is the concentration of ISIS 22023 required for half-maximal reduction of Fas mRNA, \( C \) is the concentration of ISIS 22023 in liver, and \( n \) is the sigmoidicity factor.

Integrated Pharmacokinetic/Pharmacodynamic Model. ISIS 22023 specifically binds to its target (Fas mRNA), and it is this hybrid target mRNA that will then be degraded by RNase H enzymatic activity. Indirect response models assume that the drug produces its action by stimulating or inhibiting the production or dissipation of a pharmacologic response (Dayneka et al., 1993). Therefore, ISIS 22023 binding to Fas mRNA was assumed to stimulate RNase H activity, which could induce the degradation of Fas mRNA (Fig. 1). The pharmacodynamic model is based on a scheme in which Fas mRNA is synthesized with a zero-order rate constant \( R_m \) and enzymatically cleaved by a first-order degradation rate \( K_{\text{deg}} \). ISIS 22023 activates RNase H to degrade Fas mRNA, thus increasing \( K_{\text{deg}} \). The relationship between ISIS 22023 concentration in hepatocytes and Fas mRNA levels is described in the following equation:

\[ \frac{d(mRNA)}{dt} = R_m - K_{\text{deg}} \times \left( 1 + \frac{(E_{\text{max}}) \times C^n}{EC_{50}^n + C^n} \right) \times (mRNA) \]  

(3)

where \( E_{\text{max}} \) is the maximum effect on \( K_{\text{deg}} \), \( EC_{50} \) is the concentration of ISIS 22023 required for half-maximal stimulation of RNase H, \( C \) is the concentration of ISIS 22023 in hepatocyte, \( K_{\text{deg}} \) is the Fas mRNA degradation rate constant, \( R_m \) is the synthesis rate of Fas mRNA, \( n \) is the sigmoidicity factor, and mRNA is the normalized Fas mRNA level at time \( t \).

Results

Pharmacokinetics and Suborgan Kinetics. After subcutaneous injection, ISIS 22023 appeared rapidly in plasma and declined monoeXponentially with time (Fig. 2). Maximum plasma concentration \( C_{\text{max}} \) of intact ISIS 22023 was approximately 118.6 \( \mu \)g/ml and was observed at 0.5 h after subcutaneous administration of 50 mg/kg drug. Concentrations of ISIS 22023 in plasma decreased rapidly with time and were below the limit of detection 7 h after dose administration (<0.07 \( \mu \)g/ml). Similar to other phosphorothioate oligonucleotides, the disappearance of ISIS 22023 from plasma was the result of distribution to tissues. The calculated plasma half-life actually presents a distribution half-life, not the true elimination half-life. Because phosphorothioate oligonucleotides (MOE oligonucleotides in particular, vide infra) have long tissue half-lives and are rapidly cleared from blood, their tissue levels are not accurately predicted from plasma concentrations. In addition, the assay method employed was not sensitive enough to quantitate plasma concentrations in the elimination phase. Thus, the plasma kinetics of oligonucleotides cannot be used to predict drug tissue levels nor to understand tissue clearance.

To characterize tissue kinetics, concentrations of ISIS 22023 in liver were measured directly. The concentration of ISIS 22023 in liver reached a maximum 2 days following subcutaneous administration and decreased slowly with time (Fig. 3). Using noncompartmental analysis, the half-lives calculated from the log-linear portion of the concentration versus time curves ranged from 8.8 to 18.8 days over the dose range of 10 to 50 mg/kg. The area under the concentration-time curve (AUC) for liver increased approximately 4.5-fold over a 5-fold range of doses suggesting linear kinetics, but \( C_{\text{max}} \) increased less than that predicted on the basis of dose alone (Table 2). Elimination half-lives from liver became longer with higher doses, increasing 2-fold from 10 to 50 mg/kg.

Fig. 1. Diagram of the integrated pharmacokinetic/pharmacodynamic model for ISIS 22023 in mouse liver.

Fig. 2. Plasma concentration-time profile of ISIS 22023 following subcutaneous administration of a single 50 mg/kg dose of ISIS 22023 to mice. Symbols represent observed concentrations in plasma (error bars represent standard deviation, \( n = 3 \)). The solid line represents predicted concentrations in plasma using nonlinear regression.
mg/kg dose. Taken together, these data suggest that both the uptake and the removal of ISIS 22023 from mouse liver were diminished as dose escalated.

Assessment of the kinetics of ISIS 22023 in whole liver homogenates assumes that distribution within the various cells and compartments within the liver is homogeneous. It is known that Fas expression is predominantly localized in hepatocytes (Ogasawara et al., 1993), where the mRNA would be targeted by oligonucleotides. To better understand the suborgan distribution of MOE oligonucleotides and further identify the correlation to pharmacodynamics, the pharmacokinetics within hepatocytes, Kupffer cells, and endothelial cells were studied following a single 50 mg/kg subcutaneous dose of ISIS 22023.

The time course of ISIS 22023 in different cell types was significantly different. Several trends are noticeable in the analysis of the kinetics in the individual cell types (Fig. 4). First, the highest concentration of ISIS 22023 was observed in endothelial cells, followed by the Kupffer cells. The concentration of ISIS 22023 observed in hepatocytes was the lowest. Second, the uptake by Kupffer cells appears to be more delayed than the uptake by hepatocytes or endothelial cells. As hepatocyte concentrations diminish the Kupffer cell concentrations appear to increase. These data may reflect a redistribution of oligonucleotide between the different cell types in liver. From immunohistochemistry studies with other oligonucleotides, it is clear that Kupffer cells are a repository for oligonucleotides long after dosing (M. Butler, unpublished data). The data obtained in these studies using direct quantitative analysis are consistent with that observation.

To make comparisons with whole liver concentrations of ISIS 22023, concentrations of ISIS 22023 in terms of per cell basis (ISIS 22023 molecules $\times 10^6$ per cell) was converted to micrograms of ISIS 22023 per gram of liver basis by eq. 4:

$$\text{ISIS 22023 concentration} \times \frac{\text{number of cells/g liver}}{\text{mol. wt.}} \times 1,000,000 \frac{\text{mol}}{\text{mol}}$$

where 1 g of mouse liver contains $128 \times 10^6$ hepatocytes (Kedderis and Held, 1996; Kawada, 1997) and molecular weight of ISIS 22023 is 7570 g/mol.

Hepatocytes contained the greatest amount of oligonucleotide based on concentrations per gram liver because of the greater cell population. The $\alpha$-phase half-life of ISIS 22023 in hepatocytes was 4.37 days (Table 3), which is somewhat more rapid than the $\alpha$-phase half-life obtained for whole liver, 9.85 days. (The terminal phase for liver and hepatocytes should be equivalent at equilibrium.) Thus, the pharmacokinetics of ISIS 22023 in hepatocytes is not well represented by the kinetics of the whole liver.

A two-compartment suborgan pharmacokinetic model (described in Fig. 1) was attempted with hepatocytes being the

<table>
<thead>
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<th>Parameter</th>
<th>Estimate ± S.E.</th>
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<tbody>
<tr>
<td>$T_{max}$ (day)</td>
<td>1.50 ± 0.28</td>
</tr>
<tr>
<td>$C_{max}$ (µg/g liver)</td>
<td>56.7 ± 2.4</td>
</tr>
<tr>
<td>$t_{1/2-a}$ (day)</td>
<td>4.37 ± 2.77</td>
</tr>
<tr>
<td>$t_{1/2-b}$ (day)</td>
<td>N.A.</td>
</tr>
<tr>
<td>$K_{01}$ (day$^{-1}$)</td>
<td>1.89 ± 0.68</td>
</tr>
</tbody>
</table>

N.A., not available, estimate cannot be accurately estimated.

TABLE 3

ISIS 22023 pharmacokinetics in hepatocyte following a single 50 mg/kg ISIS 22023 administered subcutaneously

Fig. 3. ISIS 22023 concentration in whole liver following subcutaneous administration of a single 10 to 50 mg/kg dose of ISIS 22023 to mice. Symbols represent observed concentrations in liver (error bars represent standard deviation, $n = 3$). The solid lines represent predicted concentrations in liver using nonlinear regression.

TABLE 2

Noncompartment analysis of ISIS 22023 in mouse liver following a single subcutaneous dose of ISIS 22023

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>AUC (µg ⋅ day/g)</th>
<th>$t_{1/2-a}$ (days)</th>
<th>$r^2$</th>
<th>$C_{max}$ (µg/g)</th>
<th>$T_{max}$ (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>374</td>
<td>8.81</td>
<td>0.992</td>
<td>29.3</td>
<td>1</td>
</tr>
<tr>
<td>20</td>
<td>799</td>
<td>11.41</td>
<td>0.980</td>
<td>48.46</td>
<td>1</td>
</tr>
<tr>
<td>35</td>
<td>1062</td>
<td>10.54</td>
<td>0.958</td>
<td>70.58</td>
<td>2</td>
</tr>
<tr>
<td>50</td>
<td>1698</td>
<td>18.80</td>
<td>0.921</td>
<td>94.07</td>
<td>2</td>
</tr>
</tbody>
</table>

Fig. 4. Concentrations of ISIS 22023 in hepatocyte, Kupffer cells and endothelial cells following single subcutaneous administration to mice in terms of number of ISIS 22023 molecules per cell. Error bars represent standard deviation ($n = 3$).
All other cell types, connective tissue and interstitium (nonhepatocyte), were assumed to comprise the peripheral compartment. Based on the experimental results, elimination of oligonucleotide from liver may be a saturable process. Michaelis-Menten elimination was attempted. However, because of the limited number of observations, the model was simplified by assuming a first-order elimination from the central compartment (Table 3).

**In Vivo Pharmacodynamics.** The pharmacologic effect of ISIS 22023 occurs in Fas-expressing cells when ISIS 22023 binds to Fas mRNA and the RNA strand of the heteroduplex is digested by RNase H (Zhang et al., 2000). Degradation of mRNA is very rapid, occurring within minutes (Wu et al., 1998). In contrast, the processes of absorption, distribution, and elimination of oligonucleotides, occur from hours to days. This difference in rates of action versus rates of delivery implies that the pharmacologic response of oligonucleotides will be limited by delivery to the targeted RNA, not enzymatic action, and predicts a direct correlation between drug concentration at the site and pharmacologic activity.

After treatment of mice with different doses of ISIS 22023, the liver Fas mRNA levels fit a typical sigmoidal dose response to the hepatocyte concentration (Fig. 5A) and to the whole liver concentration (Fig. 5B). The pharmacodynamic parameters were estimated according to the inhibitory sigmoidal $E_{\text{max}}$ model (eq. 2), and the obtained EC$_{50}$ was 16.1 $\pm$ 9.3 $\mu$g of ISIS 22023 in hepatocytes per gram of liver (Table 4).

Activation of Fas by agonist Fas monoclonal antibodies in hepatocytes triggers a proapoptotic signal transduction pathway leading to mortality from acute liver injuries. Decrease in Fas expression would be predicted to protect mice from fulminant hepatitis initiated from agonist Fas monoclonal antibodies. The relationship of Fas mRNA reduction with mortality induced by agonistic Fas antibody was characterized using a sigmoidal $E_{\text{max}}$ model. The more marked the reduction in mRNA expression the lower the mortality rate. To achieve a 50% reduction of the mortality rate, mRNA levels have to be maintained at 44% of normal level.

**Pharmacokinetic/Pharmacodynamic Relationship following Single and Multiple Administration.** The integrated pharmacokinetic/pharmacodynamic model was based on the data from a single-dose study (eq. 3). The estimated parameters are shown in Table 5. Fas mRNA levels decreased rapidly as ISIS 22023 concentration increased following subcutaneous administration. The maximum mRNA reduction was approximately 90% and occurred 2 days after a single dose of 50 mg/kg (Fig. 6), concurrent with the maximum concentration of ISIS 22023 in liver and hepatocytes. As the concentration of ISIS 22023 in hepatocytes declined, appreciable inhibition of Fas mRNA also diminished and Fas mRNA levels began to rise, presumably, because the expression of new mRNA exceeded the degradation of old Fas mRNA. By 14 days after dose administration, when concentrations of ISIS 22023 in hepatocytes were relatively low, on the order of 10 $\mu$g/g of liver, Fas mRNA levels decreased as ISIS 22023 concentration in liver increased (Fig. 6).

**Table 4**

<table>
<thead>
<tr>
<th>Parameter</th>
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</tr>
</thead>
<tbody>
<tr>
<td>$E_{\text{max}}$ (% control)</td>
<td>140.0 ± 62.7</td>
</tr>
<tr>
<td>EC$_{50}$ (µg in hepatocyte/g liver)</td>
<td>16.1 ± 9.29</td>
</tr>
<tr>
<td>$E_0$ (% control)</td>
<td>26.0 ± 12.0</td>
</tr>
<tr>
<td>$n$</td>
<td>3.97 ± 4.06</td>
</tr>
</tbody>
</table>

**Table 5**

<table>
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<th>Parameter</th>
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</thead>
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<tr>
<td>$E_{\text{max}}$</td>
<td>4.63 ± 1.94</td>
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<tr>
<td>EC$_{50}$ (µg in hepatocyte/g liver)</td>
<td>29.0 ± 5.3</td>
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<tr>
<td>$K_{in}$ (%mRNA/day)</td>
<td>87.7 ± 48.1</td>
</tr>
<tr>
<td>$K_{out}$ (day$^{-1}$)</td>
<td>0.677 ± 0.377</td>
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Pharmacokinetics and Pharmacodynamics of an Oligonucleotide

Discussion

Phosphorothioate oligonucleotides, including those with MOE-modified termini, are rapidly cleared from plasma and distribute to tissues where they produce their pharmacologic effects. As a result of their site of action, their rapid clearance from plasma, and their long half-lives in tissues, it is difficult to relate pharmacologic activity to plasma concentrations of oligonucleotide. Although antisense oligonucleotides represent a novel class of therapeutic agent, their activity may still be described in conventional pharmacologic terms. For antisense drugs, the receptor is mRNA, the binding motif is Watson and Crick base-pairing, and the ligand-receptor interactions result in destruction of the receptor (mRNA).

Studies in this laboratory and others have characterized the pharmacology of phosphorothioate oligodeoxynucleotides with MOE modifications, which represent second-generation chemistries. Structure activity relationships (i.e., sequence and mismatched sequence data) have been defined and dose-response relationships have been characterized. One significant factor missing was the correlation between oligonucleotide tissue concentrations and target organ pharmacologic effect.

In this study, we have demonstrated that there is a concordance between concentrations of oligonucleotide in liver after single and multiple doses and reductions in Fas mRNA. As concentrations of oligonucleotide in liver increase, there is a concurrent reduction in Fas mRNA, and as liver is cleared of oligonucleotide over time, there is an increase in mRNA. The Fas mRNA levels correlated with the physiological responses of mice to treatment with an antibody to Fas that binds and activates Fas to induce apoptosis and ultimately, mortality. These reductions in mRNA inversely correlated with mortality in antibody challenged mice with a conventional sigmoidal dose-response relationship. The studies presented herein went beyond relating organ concentrations to pharmacologic effect to determine target cell concentrations. A higher concordance was apparent between the concentrations in hepatocytes, Fas-expressing cells in liver, and reductions in mRNA. This difference was due to the fact that hepatocytes cleared oligonucleotide at a faster rate than other cells in liver (Fig. 4). Thus, the whole liver concentration did not accurately represent hepatocyte concentrations. For example, duration of the reduction in mRNA following single-dose administration was approximately 7 days. Between 7 and 15 days, liver concentration of oligonucleotide remained high, however, Fas mRNA levels returned back to normal.

There are several possibilities that can explain this phenomena: 1) tolerance developed following oligonucleotide treatment, or 2) whole liver was not the target, and 3) suborgan or cell-specific kinetics were critical. There does not appear to be tolerance to the drug treatment based on the responses observed in the repeat administration experiments. Each administration of ISIS 22023 induced a similar reduction in mRNA levels, suggesting that there was equivalent response with each re-administration. The appearance of the reductions in mRNA with repeated administration also suggests that there is no rebound phenomenon. The discordance between liver concentrations and response is most likely the result of differences in suborgan kinetics and drug binding to noncellular compartments. Examining hepatocyte concentrations revealed that ISIS 22023 concentration in hepatocytes dropped significantly between 7 and 15 days. These data support the concept that there is a concentration differential between whole liver and the more rapidly clearing hepatocytes, and help explain why mRNA levels begin to return to normal levels before liver concentrations have diminished appreciably and suggest that the site of ISIS 22023 action is within the hepatocyte, which is predicted based on expression.

Although, we realize that the data collected here are limited, and may overparameterize the model, this is the first study to demonstrate the pharmacokinetic and pharmacodynamic relationships of an antisense drug in vivo. Based on the estimated parameters for both the kinetic model and

![Graph](https://example.com/graph.png)

**Fig. 6.** Pharmacokinetic/pharmacodynamic relationship of ISIS 22023 in mouse liver following subcutaneous administration of a single 50 mg/kg dose of ISIS 22023 to mice. Symbols represent observed concentrations or Fas mRNA levels (error bars represent standard deviation for ISIS 22023 concentrations, or standard error for Fas mRNA levels, n = 3). Solid lines represent predicted ISIS 22023 concentrations or Fas mRNA levels using nonlinear regression.
dynamic model, a 24 mg/kg dose given once a week should provide sufficient protection of the mice subjected to agonistic Fas antibody challenge. In fact, a recent study showed that using the above simulation-based regimen, 100% protection was achieved for up to 5 months in the mice receiving ISIS 22023 treatment and subjected to agonistic Fas antibody challenge (data not shown). These studies demonstrate that the model has a good predictive power. In conclusion, this study demonstrated that: 1) concentrations in the target cells are critical to activity; 2) antisense activity follows a classic concentration-response relationship; and 3) dose regimens for this class of drugs like other classes should be designed based on concentrations at the active site or cell.

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