

PHARMACOKINETICS OF A POTENTIAL HUMAN CYTOMEGALOVIRUS THERAPEUTIC, A PHOSPHOROTHIOATE OLIGONUCLEOTIDE, AFTER INTRAVITREAL INJECTION IN THE RABBIT

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ABSTRACT:

The disposition of ISIS 2922, a phosphorothioate oligonucleotide for treatment of cytomegalovirus associated retinitis, was evaluated in rabbits. Vitreous humor and retina samples were collected from rabbits that received a single intravitreal injection of 66 μg [^{14}C]-labeled ISIS 2922 and were analyzed using anion exchange HPLC. Four hr postdosing, the concentration of ISIS 2922 in vitreous humor was 3.3 μM . The elimination of ISIS 2922 from the vitreous humor exhibited first-order kinetics with a $t_{1/2}$ of 62 hr. By 10 days postdosing, the mean concentration of ISIS 2922 in rabbit vitreous humor had decreased to 0.17 μM , which represented 22% of the total radioactivity remaining in the vitreous. The remaining 78% coeluted on anion exchange HPLC with shorter oligonucleotides. In retina, ISIS 2922 accumulated over the first 5 days postdosing, reaching a maximum concentration of 3.5 μM , and then declined thereafter with an estimated $t_{1/2}$ of 79 hr. By 10 days

postdosing when only 24% of the total radioactivity in the retina was parent compound, the concentration of ISIS 2922 remained at 1.6 μM , which was 10 times higher than the concentration in the vitreous humor. Whereas the elimination of full-length ISIS 2922 and total radioactivity from the vitreous humor occurred at nearly equal rates, ISIS 2922 disappeared more rapidly than did total radioactivity from the retina, suggesting a greater role for metabolism in the clearance process from retina than the vitreous. Alternatively, the results are consistent with metabolites being cleared from the vitreous at approximately the same rate as parent compound while in the retina metabolites may be cleared more slowly. The data were analyzed with a user-defined pharmacokinetic model, which was then used to predict the potential for accumulation of ISIS 2922 during clinical dosing.

Antisense oligonucleotides are currently being evaluated in clinical trials as anti-cancer (1), anti-inflammatory (2), and antiviral therapeutics (3). Antisense oligonucleotides are designed to hybridize to specific mRNAs, thereby inhibiting protein expression. Antisense oligonucleotides currently in clinical trials are analogues of DNA which have been modified to increase *in vivo* stability (4). Almost all antisense compounds currently being evaluated in clinical trials are phosphorothioate oligonucleotides, DNA molecules in which a single non-bridging oxygen has been replaced by a sulfur (5). Antisense approaches provide a level of selectivity not available with traditional therapeutic agents (6, 7) and have shown specific pharmacological activity in a number of animal models (8).

Human cytomegalovirus (HCMV) is a ubiquitous herpes virus which can cause severe morbidity or mortality in immunocompromised patients and is often associated with gastroenteritis and sight-threatening chorioretinitis. Currently available therapies for CMV-induced retinitis include ganciclovir (9-(1,3-dihydroxy-2-propoxymethyl)guanine) and foscarnet (phosphonoformate). The use of these compounds is limited by the emergence of resistant strains of HCMV, indicating the need for new drugs or treatment strategies.

ISIS 2922 is a phosphorothioate oligonucleotide, 21 nucleotide units in length, which has shown antiviral activity against cytomegalovirus *via* inhibition of expression of the major immediate early gene (9). It is currently being evaluated in clinical trials for activity against CMV-induced retinitis. All currently available therapies for

CMV retinitis target the cytomegalovirus DNA polymerase, and thus resistant strains to one therapy may be resistant to other therapeutic agents that target the same enzyme. ISIS 2922 inhibits the expression of a pivotal replication protein, one of the immediate early genes (9). Thus, ISIS 2922 inhibits CMV replication at a different target than currently available therapies, and viruses resistant to those therapies do not show cross-resistance to ISIS 2922 (10). ISIS 2922 targets a 21 nucleotide sequence in the coding region. Single-base mismatches in the oligonucleotide reduce the activity only slightly (11). It is, therefore, unlikely that resistant strains of CMV will be selected by this treatment. Hence, ISIS 2922, as an antisense oligonucleotide therapeutic, not only has a different molecular target than currently available CMV-retinitis therapies, but also may be less likely to result in the rapid emergence of resistance (11), making it useful as a therapeutic alternative, especially in populations of patients where resistance to other therapies has occurred.

Although there have been numerous investigations into the systemic pharmacokinetics of phosphorothioate oligonucleotides (4, 5, 12–14), none have described the pharmacokinetics of an intravitreally administered phosphorothioate oligonucleotide. *iv* administration of a [^{14}C]-radiolabeled phosphorothioate oligonucleotide did not lead to any appreciable radiolabel in the eye (12). Therefore, to achieve therapeutic concentrations in the eye an alternative route of administration had to be established. The goal of this study was to characterize the disposition of ISIS 2922 after intravitreal injection and, in particular, to determine the persistence of the parent drug, ISIS 2922, in the vitreous humor and retina. Rabbits were given a single intravitreal injection of [^{14}C]-labeled ISIS 2922 in the left eye, and tissues were harvested at various times after dosing for analysis of the concentra-

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tion of remaining ISIS 2922 as well as the identification of metabolites. Additionally, the level of residual radioactivity was measured in internal organs that had been shown to be sites of accumulation of oligonucleotide after systemic administration.

Materials and Methods

Synthesis of Radiolabeled Oligonucleotide. ISIS 2922 is a phosphorothioate oligonucleotide composed of 21 nucleotide units of the following sequence: 5'-GCGTTTGTCTTCTTCTTTCGCG-3'. [¹⁴C]-Radiolabeled ISIS 2922 was synthesized according to a previously published procedure (15). The [¹⁴C]-label was incorporated in the C-2 position of the thymine base, of which there are 10 in ISIS 2922. The specific radioactivity of the oligonucleotide was 12.5 μ Ci/mg for all oligomers, and the purity of the material was approximately 75%, as defined in terms of full-length oligomers with minimal phosphodiester content. The remaining radiolabeled material consisted primarily of chain-shortened oligonucleotide impurities, coeluting on anion-exchange HPLC with oligonucleotides shorter than full length ISIS 2922 by 2 or 3 nucleotides. The pharmacokinetics of phosphorothioate oligonucleotides has been shown to be independent of the purity, in terms of full-length oligonucleotide (unpublished observation).

In-Life Treatment. Rabbits were given a single intravitreal injection of [¹⁴C]-ISIS 2922 (Lot No. ISIS-0026) in the left eye, and tissues harvested at various times post dosing ($N = 2-3$ animals/time point). The oligonucleotide was formulated in a sodium bicarbonate buffer, pH 8.7. The injection volume was 0.1 ml, and the dose per eye was 0.84 μ Ci of radioactivity and 66 μ g of oligonucleotide. Assuming a vitreous volume of 1.5 ml for rabbits, the estimated initial vitreal concentration achieved by the dose given was approximately 6.2 μ M (based on all oligomers) or 4.65 μ M (full-length ISIS 2922).

Sample Processing and Storage Conditions. Tissue samples were processed and evaluated for [¹⁴C] activity. Each sample was weighed and oxidized (Packard model 306 oxidizer) prior to analysis for [¹⁴C] activity in a Packard Liquid Scintillation Counter (Minaxi Tri-Carb 4000 Series, using Packard scintillation cocktails, Meriden, CT). For those samples too large to be evaluated *in toto*, as well as those samples reserved for analysis of oligonucleotide content, a representative sample was evaluated. If any of the tissues weighed less than 0.5 g, one-half was reserved for HPLC analysis. A 50- μ l or representative sample of the vitreous humor was processed for isotope activity, with the remainder analyzed by HPLC. Retinas were dissected from the choroid under a dissecting microscope. Careful dissection procedures were followed to ensure no vitreous humor remained adhered to the retina. To facilitate dissection, eyes were frozen up to 10 min on dry ice.

Extraction of Samples Prior to Chromatography. For retina samples, protein was digested by first incubating each sample in a 2.0 mg/ml solution of proteinase K containing 0.5% Non-Idet P-40 (NP-40) with 20 mM Tris-HCl (pH 8.0), 20 mM EDTA, and 100 mM NaCl at 37°C for 16–24 hr. Two phenol-chloroform (1:1; v/v) extractions followed by one chloroform extraction were used to remove proteinase K, digested protein, and lipids from the nucleic acids. After phenol-chloroform extraction, the samples were evaporated to dryness in a Savant Speedvac SC100 (Farmingdale, NY) and resuspended in distilled H₂O. Vitreous humor samples were extracted in the same manner except that the proteinase K was added directly to the vitreous humor without extraction buffer. This method was adapted from that used by Agrawal *et al.* (5).

Strong Anion Exchange (SAX) High Performance Liquid Chromatography (HPLC). Aliquots from the extracted retina and vitreous humor samples were analyzed by HPLC using a Gen Pak Fax strong anion exchange chromatography column (4.6 mm, i.d.; 100 mm, l; Waters, Milford, MA). Intact [¹⁴C]-ISIS 2922 was separated from metabolites using the following mobile phase and gradient: Buffer A: 86 mM Tris-HCl (pH 8.0), 20% methanol; Buffer B: 86 mM Tris-HCl (pH 8.0), 1.5 M NaBr; Gradient: 100% A isocratic for 5 min, then linear to 60% B over 45 min at a flow rate of 0.5 ml/min. Eluate was collected from the HPLC in separate vials at the rate of one/min, and 5 ml of ReadySafe scintillation cocktail (Beckman Instruments, Fullerton, CA) was added for quantitation of radioactivity in a LS3801 liquid scintillation counter (Beckman Instruments, Fullerton, CA).

Calculation of ISIS 2922 Concentration. The SAX-HPLC is capable of resolving the full-length, fully thiated oligomer (21-mer $P = S$) from oxidized

structures (*e.g.* oligomers containing one or more phosphodiester linkages in the backbone) as well as most other impurities. However, the SAX-HPLC system does not effectively separate full-length (21-mer) oligonucleotide from $n-1$ (*e.g.* 20-mer) impurities/metabolites. Thus, ISIS 2922, as discussed here, refers to the percentage of radiolabel which, on HPLC, coelutes with intact ISIS 2922 and would include metabolites shortened by one oligonucleotide (the 20-mer). The percentage of radioactivity which coeluted with ISIS 2922 on HPLC was multiplied by the micromolar equivalents for the sample. This was used as the concentration of ISIS 2922.

Pharmacokinetic Analysis and Modeling. Multiple dose kinetics of ISIS 2922 in vitreous and retina were simulated using a pharmacokinetics modeling program (16). A model assuming linear kinetics with instantaneous input into the first compartment (*i.e.* vitreous) was used to simultaneously regress on observed concentrations in the vitreous and retina after a single dose as described above. Equilibrium was assumed between the vitreous and retina and between the retina and another tissue compartment, with elimination from the vitreous and retinal compartments. Correlation between this model and the data was greater than 0.9, the minimum correlation considered a good fit. Multiple intravitreal doses of ISIS 2922 were simulated using weekly or every other week administration. Log-linear regressions were used for the calculation of elimination half-lives. All points were included for analysis of the elimination half-lives from vitreous, while, for retina, only the time points starting at 120 hr and later were used (table 1). Additionally, for retina, two time points that appeared to be outliers (168 and 288 hr) and were derived from single sample analysis were excluded from the curve fitting and modeling process.

Results

Systemic Exposure and Elimination. The levels of radioactivity in the blood and plasma were negligible, with values very near background radioactivity. The levels of ISIS 2922-derived radioactivity in internal organs and tissues such as kidneys, liver, and skin were also very low relative to ocular tissue levels. The highest concentration of total radioactivity in a non-ocular tissue was approximately 30 nM-equivalents, seen in the kidney at 24 hr after the injection. The cumulative amount of the radioactivity excreted in the urine and feces over the 20-day collection period was 16.7 and 3.2% of the administered dose, respectively. Given the low levels of radioactivity in urine, the HPLC assay would not have provided adequate sensitivity to detect oligonucleotide. Although samples were not analyzed for the amount of intact oligonucleotide, previous studies in rats with higher systemic doses of other phosphorothioate oligonucleotides showed no intact oligonucleotide in urine (12, 17).

Strong Anion Exchange HPLC Analysis. Based on examination of various monomer and oligomer standards, radioactivity eluting at the void volume likely represents a mixture of mono- and dinucleotides, nucleosides and bases, while the radioactivity eluting between 20 and 45 min on the SAX-HPLC represents shorter oligonucleotides formed by nucleolytic hydrolysis of ISIS 2922. Chromatograms from vitreous and retinal extracts from 240 hr post-injection figs. 1 (A) and (B) contained radioactivity that coeluted with low molecular weight metabolites, chain-shortened oligonucleotide metabolites, as well as intact ISIS 2922. The vitreous and retinal extracts contained the same metabolites although the relative proportions were different. Over the time course of the experiment the amount of total radioactivity as well as the relative amount of full length oligonucleotides decreased in vitreous humor and retina.

Concentration and Kinetics of Radioactivity and ISIS 2922 in Vitreous. The clearance of radioactivity from the vitreous exhibited first-order kinetics with a half-life of approximately 3 days (77 hr). Radioactivity appeared in the aqueous humor within 4 hr postdosing, but the level was consistently approximately 10% of that in the vitreous humor. At 4 hr postdosing, the vitreal concentration of ISIS 2922 was 3.31 μ M, which decreased to 0.02 μ M by 20 days (table 1). The elimination half-lives for total radioactivity and intact ISIS 2922

TABLE 1

Concentration of ISIS 2922 and total radioactivity in vitreous and retina after a single intravitreal injection

Time Post-Injection (hr)	Vitreous			Retina		
	μM -Equivalent	Fraction Coeluting on HPLC with Intact	ISIS 2922 (μM)	μM -Equivalent	Fraction Coeluting on HPLC with Intact	ISIS 2922 (μM)
4	5.61	0.59	3.31	3.0	0.48	1.46
24 ⁱ	4.04 \pm 0.17	0.47 \pm 0.03	1.89 \pm 0.19	5.37 \pm 0.22	0.42 \pm 0.03	2.24 \pm 0.16
72	3.71	0.34	1.28	9.23	0.29	2.79
120	2.96	0.40	0.75	9.43	0.31	3.51
168	0.90	0.27	0.24	2.7	0.15	0.41
240	0.75 \pm 0.065	0.22 \pm 0.16	0.17 \pm 0.13	6.5 \pm 0.62	0.24 \pm 0.03	1.56 \pm 0.27
288	0.42	0.29	0.12	1.65	0.14	0.14
336	0.31	0.03	0.01	1.98	0.23	0.46
384	0.17	0.13	0.02	2.62	0.17	0.25
432	0.22 \pm 0.045	0.18	0.04 \pm 0.03	2.90 \pm 1.22	0.18 \pm 0.04	0.54 \pm 0.28
480	0.18	0.08	0.02	1.22	0.09	0.11

ⁱSDs are shown for the three time points, 24, 240, and 432 hr, which had three animals.

The concentration of [¹⁴C] derived from [¹⁴C]-radiolabeled ISIS 2922, the percent of the radiolabel coeluting with intact ISIS 2922 on HPLC, and the concentration derived by multiplying those two parameters, are shown over time after a single intravitreal injection of 66 μg of [¹⁴C]-ISIS 2922.

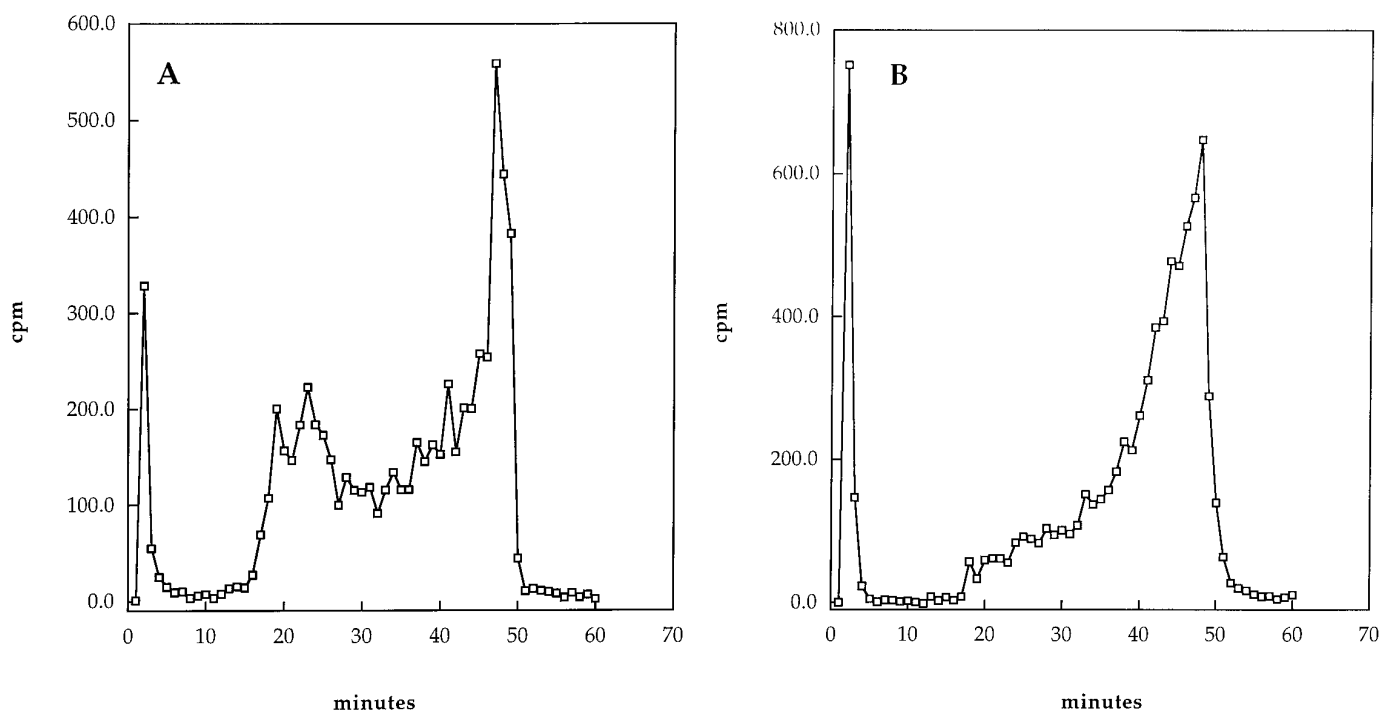


FIG. 1. HPLC oligonucleotide metabolite profile. Representative radiochromatograms of vitreous sample (A) or retinal sample (B) obtained at 240 hr after intravitreal injection.

Radioactivity in 1 min fractions eluted from the SAX-HPLC column. Parent compound, ISIS 2922, elutes at 45 min.

were similar, with estimates of 77 and 62 hr, respectively. Over time, ISIS 2922 concentrations and total radioactivity were nearly parallel, suggesting that both metabolites and parent compound were cleared at similar rates.

Concentration and Kinetics of Radioactivity and ISIS 2922 in Retina. The concentration of radioactivity in the retina increased over the first 5 days postdosing, reaching a maximum of 9.43 μM equivalents, assuming a density of 1.0 for the retina (table 1). The [¹⁴C] levels in the retina slowly decreased thereafter with an estimated half-life of 164 hr. Beyond 4 hr postdosing, the levels of radioactivity

in the retina exceeded the levels in the vitreous humor. By 120 hr postdosing, the retina/vitreous ratio for total radioactivity was 4.7 fold, and this ratio tended to increase during the remainder of the 20-day study period owing to the somewhat faster clearance of radioactivity from the vitreous ($t_{1/2} = 3$ days), relative to the retina ($t_{1/2} = 6.8$ days). The level of radioactivity in the iris was comparable with that in the retina, whereas the amount of radioactivity in the optic nerve was very low throughout the study, with less than 0.1% of the administered radioactivity localized there throughout the time course of the study. A number of ocular tissue samples from the same time

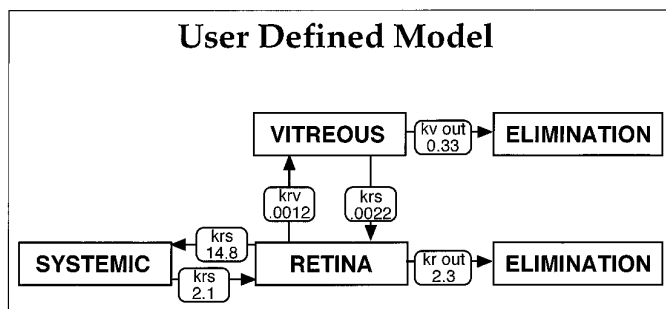


FIG. 2. Pharmacokinetic model.

The user-defined model which illustrates the theoretical compartments and kinetic constants potentially involved in the flow of oligonucleotide after intravitreal injection. The units for the values for the rate constants derived from nonlinear regression analysis are Days⁻¹.

points had greater than two-fold differences in their levels of radioactivity between eyes. Because the total radioactivity data were used to calculate the concentration of ISIS 2922, this variability contributed to the variability in the levels of intact oligonucleotide seen in the retina.

The concentrations of ISIS 2922 in retina rose for 5 days and then tended to slowly decrease over the observation period; analogous to the time curve of total radioactivity. ISIS 2922 increased in the retina for several days after the intravitreal injection (table 1). The observed peak concentration of ISIS 2922 occurred 5 days after intravitreal injection, at which time the concentration was 3.5 μM . Subsequently, the levels began to decline with a half-life of 79 hr. Intact ISIS 2922 represented from 48.7% to 8.4% of the total radioactivity over the 20 days of the experiment. The low percentage of intact oligonucleotide at later time points suggests that metabolism within the retina is an important component of the clearance process. Alternatively, it is possible that metabolites of ISIS 2922 are eliminated more slowly from the retina than ISIS 2922 which may, in part, account for the diminishing percentage of total radioactivity represented by full length oligonucleotide and for the longer $t_{1/2}$ of total radioactivity, 164 hr versus 79 hr for intact ISIS 2922. By 480 hr, or 20 days after the injection, the level of intact ISIS 2922 was 0.11 μM . Although it is not possible to say what concentration may be required *in vivo* for activity, this observed concentration is still within the range associated with antiviral activity *in vitro*.

Pharmacokinetic Modeling. Optimal conformance to the data was achieved simultaneously for both vitreous and retinal concentrations of intact drug. A mathematical model assuming first-order kinetics with exchange between vitreous and retina, as well as between retina and a systemic vascular compartment, was fit to the data (See fig. 2 for model and fig. 3 for fit). Elimination of intact material from the vitreous and retina (including metabolism) was also accounted for in the model. Clearance from the vitreous was estimated to be 0.97 ml/day, and the derived distribution volume was 3.6 ml. Given that the estimated volume of rabbit vitreous is 1.5 ml, the oligonucleotide does not appear to be widely distributed. The rate constants for the model that was obtained from the regression are shown in fig. 2. The correlation coefficient between the model and the data was 0.95.

The model determined for a single intravitreal dose of ISIS 2922 was used to predict ocular exposure after repeat dosing. The dose regimen modeled was once weekly injection of ISIS 2922 for 4 weeks, followed by every-other-week maintenance dosing (assuming a dose which would result in a peak intravitreal concentration of approximately 4 μM). According to the model, the pharmacokinetics of intact ISIS 2922 in the vitreous humor approaches steady state within the

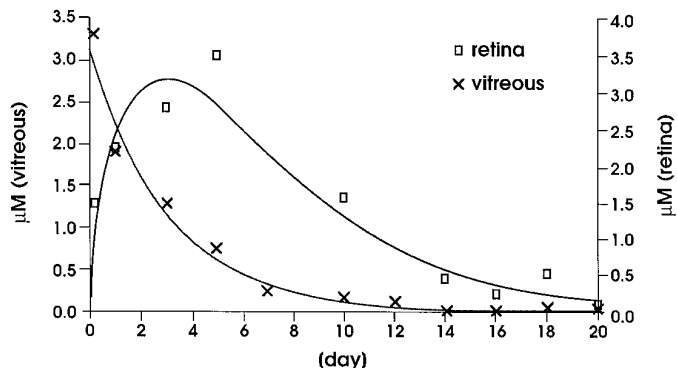


FIG. 3. Comparison of data with model.

This figure illustrates the fit of the model to the concentrations of intact ISIS 2922 in retina, excluding two outlier points, and in vitreous humor.

first 2–3 weeks, with a C_{max} that is only slightly higher than the single-dose C_{max} . The model suggests that there is little potential for accumulation of intact ISIS 2922 in the vitreous with this dosing schedule. In contrast, owing to the initial accumulation phase in the retina and the slow subsequent clearance of ISIS 2922, weekly dosing would be expected to result in moderate accumulation of intact ISIS 2922 in the retina. Specifically, the C_{max} achieved after 4 weekly doses was predicted to be 66% higher than the peak concentration after a single dose. Moreover, the concentration of intact drug in the retina would stay within a fairly narrow concentration range of 2–5.0 μM during the first month. With every other week administration, little or no accumulation of intact ISIS 2922 would be predicted to occur in the retina, and the concentrations of intact ISIS 2922 would range from 3.7 to 0.6 μM for the remainder of the treatment period.

Discussion

Based on the results obtained in this study, ISIS 2922, a phosphorothioate oligonucleotide intended for treatment of HCMV, has a potentially useful pharmacokinetic profile when given by intravitreal injection, owing to the accumulation and persistence of intact drug in the target tissue, retina. Four hr after administration, the concentration of ISIS 2922 in vitreous humor was 3.3 μM . Vitreous levels decreased slowly, and elimination appeared to be first-order. The vitreous concentration remained above 0.10 μM , where antiviral activity is observed *in vitro*, for 288 hr after administration. In the retina, the concentration of intact ISIS 2922 increased during the first days after intravitreal administration, reaching the maximum observed concentration of 3.5 μM 5 days after intravitreal injection. Subsequently, there was a slow nonlinear decline in the retinal ISIS 2922 concentration. By 480 hr after administration, intact ISIS 2922 concentration in the retina was 0.17 μM , which is still within the range of concentrations shown to inhibit 90% of virus replication in cell culture assays (9). The elimination of ISIS 2922 from vitreous and retina appears to be owing, at least in part, to metabolism. Anion exchange HPLC analysis showed evidence of chain-shortened metabolites, likely formed by nucleolytic cleavage. This has been reported as a mechanism of metabolism for a number of other phosphorothioate oligonucleotides (8, 18). The slow elimination of ISIS 2922 may allow for infrequent dosing in clinical studies.

The estimated half-lives for ISIS 2922 and total radioactivity in the vitreous were 62 and 77 hr, respectively, suggesting metabolism of the oligonucleotide plays a minimal role in clearance of oligonucleotide from vitreous. Inulin, a nonmetabolizable polar polysaccharide of 5000 Da molecular weight has a reported $t_{1/2}$ of 36 hr after intravitreal

administration to rabbits and is cleared primarily *via* outflowing aqueous humor (19). Intravitreally-administered inulin is believed to represent the extreme of slow ocular clearance. It is unclear whether the higher molecular weight of ISIS 2922, approximately 7000 Da, or some affinity for an unknown component of vitreous is responsible for the relatively slower clearance. Smaller molecules being evaluated for the treatment of human CMV retinitis which are being administered directly into the vitreal compartment have been reported to also have shorter half-lives in the vitreous. For example, the estimated elimination half-life for (S)-1-(3-hydroxy-2-phosphorylmethoxypropyl) cytosine was reported to be 18 hr (20). This value is much shorter than the reported $t_{1/2}$ for inulin. Trifluorothymidine, evaluated as a potential intravitreal therapy against CMV-induced retinitis (21), exhibited first-order elimination kinetics from vitreous after intravitreal injection in New Zealand white rabbits with a $t_{1/2}$ of only 3.15 hr. The elimination half-life of foscarnet after intravitreal administration was 34 hr in rabbits (22) and 54 hr in humans (23). Thus, ocular elimination of foscarnet was very similar to that reported for inulin.

The estimated $t_{1/2}$ for ISIS 2922 in retina was more than two-fold shorter than that estimated for total radioactivity. Together, the low percentage of total radioactivity that coeluted on HPLC with intact ISIS 2922, the high percentage of metabolites, all suggest that metabolism was more rapid in retina than clearance of the metabolites. Unfortunately, unlike the present study, none of the previous studies of intravitreal pharmacokinetics of therapeutic compounds reported corresponding retinal levels. Therefore, comparisons of retina disposition from the present study with previous studies could not be made.

Systemic exposure after intravitreal injection of ISIS 2922 was assessed by measurement of radioactivity in blood and non-ocular tissues. The analytical methods used were not sensitive enough for detection of intact oligonucleotide given the low levels of radioactivity in non-ocular tissues. Among these tissues, the highest concentrations of radioactivity were found in kidney and liver, which are known sites of accumulation for phosphorothioate oligonucleotides after systemic administration (12). Based on the peak levels of radioactivity in these organs, the maximum possible concentration of oligonucleotide would be no more than 30 nM. Thus, the systemic exposure after intravitreal administration was very low and would not be expected to produce any systemic effects.

To design a clinical dosing regimen such that adequate oligonucleotide concentrations are maintained in vitreous and retina without causing significant accumulation in either location, the concentrations of ISIS 2922 measured after a single intravitreal injection in New Zealand white rabbits were used in a pharmacokinetic model to predict levels achieved after 4 weekly injections followed by every other week administration of the same dose. ISIS 2922 would not be expected to accumulate to a significant degree in the retina (steady-state levels projected to be 66% higher than a single injection when given on a weekly basis) and would not be likely to accumulate at all in the vitreous. The concentration of ISIS 2922 in the retina was predicted to range from 5.0 to 0.6 μ M throughout the treatment period, which, based on *in vitro* potency, should be sufficient to maintain continuous anti-CMV activity (9). Thus, the results of this study suggest that, if the pharmacokinetics are similar in humans, intravitreally administered ISIS 2922 would remain at concentrations at which antiviral activity has been observed *in vitro*.

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