Evaluation of Retinal Toxicity and Efficacy of Anti-Cytomegalovirus and Anti-Herpes Simplex Virus Antiviral Phosphorothioate Oligonucleotides ISIS 2922 and ISIS 4015

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Retinal toxicity of ISIS 2922 and ISIS 4015, phosphorothioate oligonucleotides complementary to human cytomegalovirus (CMV) and herpes simplex virus (HSV) RNA, were evaluated. The intravitreal concentration of ISIS 2922 found not to cause permanent toxic changes in the rabbit retina was 10 μM and in the pig retina, 5 μM. The 3 μM concentration was associated with a transient inflammatory response, and 1 μM caused no retinal toxicity or inflammation. ISIS 4015 showed very mild toxicity with no permanent retinal changes and very mild inflammation at doses of 10 μM; this dose was effective in ameliorating or preventing HSV-1 retinitis when injected 1 day and 1 week prior to virus inoculation. These oligonucleotides have a low intraocular therapeutic index. Attempts to improve the therapeutic index of these compounds are indicated. Only a clinical trial can determine the toxicity profile of ISIS 2922 for the treatment of CMV retinitis.

Antisense oligonucleotides have been shown to act as specific inhibitors of gene expression in a variety of in vitro systems. They are considered a promising new generation of drugs, potentially useful in most human diseases, including cancers and viral infections [1].

Therapeutic applications of antisense oligonucleotides have been restricted by a number of difficulties, including stability, pharmacokinetic behavior (both on the cellular and systemic level), and the high cost of industrial production [2]. One of the advantages of intravitreal delivery of antisense agents for retinal disease is the access of the compounds to infected tissue and the fact that the vitreous is not known to enzymatically destroy these compounds, which should allow longer survival of the intact antisense compound [3].

Significant interest exists in the use of locally administered antiviral drugs given by intravitreal injection for viral retinitis, particularly cytomegalovirus (CMV) retinitis in AIDS patients because of toxicity (which may be reversible) and development of resistance to systemic anti-CMV drugs [4, 5]. Antiviral resistance to ganciclovir or foscartern has been described in patients taking these medications for several months or longer, and progression of retinitis is common. Intravitreal injections of these two antiviral drugs can be safely performed, but they are poorly tolerated by patients because of the need of frequent injections [6, 7]. Recently an intravitreal sustained-release ganciclovir implant has been developed with promising results [8].

We evaluated the intraocular tolerance of ISIS 2922, a phosphorothioate oligonucleotide complementary to human CMV (HCMV) RNA in rabbit and pig eyes and the intraocular tolerance of ISIS 4015, a phosphorothioate oligonucleotide complementary to herpes simplex virus type 1 (HSV-1) RNA in rabbit eyes. The highest nontoxic intravitreal concentration was determined. To test the hypothesis that oligonucleotides can be used to treat viral retinitis via intravitreal injection, we studied the effect of ISIS 4015 in an experimental model of induced HSV-1 retinitis. Because there is no appropriate animal model for HCMV retinitis, we did not evaluate the efficacy of ISIS 2922 [9]. The in vitro specificity and potency of ISIS 2922 suggest that it may be useful for the treatment of HCMV [10].

Materials and Methods

The phosphorothioate oligonucleotides, ISIS 4015 and ISIS 2922, were synthesized on a solid-phase DNA synthesizer as described [11]. One lot of ISIS 2922 drug substance was purified by preparative reversed-phase chromatography, anion-exchange–negative (SAX−). Two other lots of ISIS 2922 drug substance were further purified by preparative strong anion-exchange chromatography (SAX+).

Of the two lots that were purified by SAX, one lot was later found to be contaminated (8% of the preparation) with another sequence, which has a toxicity profile that may be different from that of a single-stranded phosphorothioate oligonucleotide. The contaminating oligonucleotide was an 8-nucleotide sequence (8 mer) that formed a tetramer in solution. This lot of drug is referred to as SAX+ oligo cont. The second lot of drug that was purified...
DNA replication. It has an EC 50 against HSV-1 (KOS strain) of histologic appearance and 4 indicated severe destruction and disor-

... 4015 at 10

... 0.37 ± 0.26 µM [10], and the mean EC90 against HCMV is scribed [15, 16]. Inflammation and toxicity were scored histologically by grading intraocular inflammation (primarily vitritis) and the degree of retinal inflammatory and optic nerve head infiltration on a 0–4 scale. In addition, permanent structural toxicity itself was graded by the degree of destruction of the intraocular structures, primarily retina, on a scale of 0–4: 0 indicated a normal retinal histologic appearance and 4 indicated severe destruction and disorganization of all retinal layers.

... eyes of 40 New Zealand White rabbits were used for toxicity studies, and 29 eyes of 39 Dutch pigmented rabbits were used for therapeutic studies. Body weights were 2.2–2.8 kg/ani-

... 4015 or with only HSV-1 (control eyes). For the toxicity studies of ISIS 2922 and ISIS 4015, both eyes of each animal were used and injected either with drug or with BSS. Anesthetic and injection were done as we have described [15, 16]. Therapeutic studies entailed evaluation of the antiviral effect of ISIS 4015 in an HSV-1 retinitis rabbit model. The species, type of ISIS compound, final intravitreal concentration, and quantity of animals used are summarized in table 1.

Toxicity studies of ISIS 2922 and 4015. All doses used are expressed in final intravitreal concentration (micromolar). The final intravitreal concentration following a 0.1-mL injection was calcu-

... 1 retinitis rabbit model. The species, type of ISIS compound, final intravitreal concentration, and quantity of animals used are summarized in table 1.

Toxicity studies of ISIS 2922 and 4015. All doses used are expressed in final intravitreal concentration (micromolar). The final intravitreal concentration following a 0.1-mL injection was calculated assuming the volume of the rabbit eye to be 1.4 mL and the volume of the pig eye to be 3.0 mL. ISIS 2922 doses of 1, 3, and 10 µM (SAX- oligo cont), 40 µM (SAX+ ref and SAX+), and 160 µM (SAX-) were evaluated in rabbits. Half of the eyes studied with 10, 40, and 160 µM (all SAX+) received a second injection 1 week after the first. Doses of 5 µM (SAX+ oligo cont) and 20 µM (SAX+ ref) of ISIS 2922 were evaluated in pig eyes. In addition, we compared an anion-exchange–purified form (SAX- oligo cont) and a nonpurified form (SAX+) of ISIS 2922 at doses of 1, 3, and 10 µM in rabbit eyes (table 1). Four intravitreal doses of ISIS 4015 (10, 32, 100, and 320 µM) were evaluated in rabbit eyes.

Both eyes of each rabbit received 0.1 mL of different concentra-

... 0.1 mL of pentobarbital sodium (390 mg/mL). Both eyes were examined with slit lamp and indirect ophthalmoscopy or with HSV-1 and ganciclovir (571 µM) at one of three concentrations (3, 10, and 32 µM). In addition, the 10 µM dose was also injected intravitreally at various times before virus inoculation to determine the duration of antiviral effect. These animals were compared. Controls were rabbits in whom only 1 eye was injected with HSV-1 and no oligonucleotides or with HSV-1 and ganciclovir (571 µM) final intravitreal concentration) 1 day before virus inoculation. HSV-1 (10,000 U) was inoculated directly onto the retinal surface as described [15, 17]. Both eyes were examined with slit lamp and indirect ophthalmoscopy on days 1, 3, 5, 7, 10, 14, 21, and 28 after HSV-1 inoculation. Diagrams and selected fundus photographs were obtained. Animals were anesthetized as described above and euthanized with an intra-cardiac injection of 1.0 mL of pentobarbital sodium (390 mg/mL).
Table 1. Experimental designs and results of toxicity study.

<table>
<thead>
<tr>
<th>No. of eyes receiving Final intravitreal concentration (µM)</th>
<th>Mean score* (1 injection/2 injections)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no. of eyes</td>
<td>Structural toxicity</td>
</tr>
<tr>
<td></td>
<td>Inflammation</td>
</tr>
<tr>
<td><strong>ISIS 2922</strong></td>
<td></td>
</tr>
<tr>
<td>Rabbit²</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>160</td>
</tr>
<tr>
<td>7</td>
<td>40 (SAX⁺)</td>
</tr>
<tr>
<td>2</td>
<td>40 (SAX⁻)</td>
</tr>
<tr>
<td>4</td>
<td>10 (SAX⁺ OC)</td>
</tr>
<tr>
<td>8</td>
<td>10 (SAX⁻)</td>
</tr>
<tr>
<td>4</td>
<td>3 (SAX⁺ OC)</td>
</tr>
<tr>
<td>4</td>
<td>3 (SAX⁻)</td>
</tr>
<tr>
<td>4</td>
<td>1 (SAX⁺ OC)</td>
</tr>
<tr>
<td>4</td>
<td>1 (SAX⁻)</td>
</tr>
<tr>
<td>8</td>
<td>BSS</td>
</tr>
<tr>
<td>Pig</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>20 (SAX⁺)</td>
</tr>
<tr>
<td>1</td>
<td>5 (SAX⁺ OC)</td>
</tr>
<tr>
<td>1</td>
<td>BSS</td>
</tr>
<tr>
<td><strong>ISIS 4015</strong></td>
<td></td>
</tr>
<tr>
<td>Rabbit</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>320</td>
</tr>
<tr>
<td>7</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>32</td>
</tr>
<tr>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>BSS</td>
</tr>
</tbody>
</table>

NOTE. Animals were sacrificed half at 2 weeks and half at 6 weeks. OC, material was 92% pure ISIS 2922 and 18% contaminated by another oligonucleotide; BSS, balanced salt solution.

* Grading of results on scale of 0–4, where 0 indicates no inflammation or toxicity and 4 indicates severe anterior and posterior segment inflammation and retinal toxicity (as described in text), based on funduscopic and slit lamp examination as well as histologic assessment.

¹ Some rabbit eyes received 2nd injection 1 week after 1st injection.

² Ultrapure preparations (SAX⁺ ref).

dehyde. Each eye was embedded in paraffin for sectioning, followed by hematoxylin-eosin staining.

Immunostaining was performed in all eyes in which a hazy vitreous precluded a good view of the retina, making the visual assessment of infection difficult. A horseradish peroxidase–conjugated rabbit anti–HSV-1 polyclonal antibody was used (Dako, Carpinteria, CA) and counterstained with hematoxylin. Noninfected rabbit retinas served as negative controls; positive controls consisted of human brain tissue from a patient with herpes simplex encephalitis. Table 2 summarizes the experimental dosing of the HSV-1 retinitis prophylactic treatment study and the results.

Retinitis was graded by 2 examiners using masked ophthalmoscopy, slit lamp examination, and histology as reported [15, 18] by a scoring vitritis, retinal opacification, and congestion of the optic nerve and medullary ray on a scale of 0 (no disease) to 4 (eyes followed the natural course of nontreated HSV-1 retinitis in our model, with initial development of papillitis and vitritis, followed by retinitis expanding to involve the entire retina by day 14) [15, 17].

Results

Toxicity studies of ISIS 2922 and ISIS 4015. Toxicology of ISIS 2922 was studied as noted above. The results of the toxicityology studies on the rabbit eyes injected with 160 µM showed severe vitritis and total retinal destruction due to retinitis at 2 weeks and at 2 months. Studies of the eyes injected with 40 µM (SAX⁺) ISIS 2922 showed moderate to severe vitritis, exudative retinal detachment, and severe optic disk and medullary ray edema that was more severe with a second injection. The eyes injected with 40 µM ISIS 2922 ultrapure form (SAX⁺ ref) showed clinically moderate vitritis, mainly over the medullary ray and optic disk, and a normal retinal appearance; histologically there was a mild to moderate amount of inflammatory cells in the vitreous over the medullary ray and a mild destruction of photoreceptor outer segments.

The 10 µM dose (SAX⁻) showed clinically mild to moderate (grade 1.5–2) destruction, inflammation in the vitreous (mainly over the medullary ray and optic disk), normal retinal appearance, and mild to moderate optic disk and medullary ray edema. Histologically there were a moderate number of inflammatory cells in the vitreous, moderate destruction of the outer nuclear layer, and moderate to severe destruction of photoreceptors (figure 1). However, eyes dosed with 10 µM (SAX⁺ oligo cont) showed clinically mild inflammation in the vitreous and there
was no evidence of retinal toxicity (figure 2). The changes were seen at 2 weeks and were less severe at 8 weeks. With two injections of SAX– compound 1 week apart, more severe inflammatory infiltration in the vitreous was seen and the outer retina was severely destroyed, including the outer nuclear layer and photoreceptors.

The 3 μM dose (either SAX– or SAX+ oligo cont) showed clinically minimal inflammatory cells in the vitreous and a normal retinal appearance. Histologically there were few (none to mild) inflammatory cells in the vitreous and a normal retina. The 1 μM dose (either SAX– or SAX+ oligo cont) showed no inflammation or toxicity. Therefore, with regard to retinal toxicity, the no-effect level was 10 μM in the rabbit, even with (SAX+ oligo cont) material.

Studies in the pig eye injected with ISIS 2922 at 5 μM final intravitreal concentration (SAX+ oligo cont) showed no inflammatory cells in the vitreous and no retinal toxicity. The eyes injected with the ultrapure form (SAX+ ref) at 20 μM showed clinically a severe inflammatory response (grade 4) and histologically a severe disorganization of the retina and numerous inflammatory cells in the vitreous.

Toxicity studies of eyes injected with ISIS 4015 showed a normal clinical appearance at 2 weeks and a normal retinal histology in control eyes and with the 10 μM dose. This was confirmed by electron microscopic examination. With the 32 μM dose, 2 of 3 eyes showed normal retinal histology and 1 of 3 had mild photoreceptor damage. With the 100 μM dose, all eyes showed moderate to severe outer retinal destruction, and with the 320 μM dose, all eyes showed total retinal destruction (figure 3). At 2 months, all the eyes with a 10 or 32 μM dose exhibited normal retinal histology and minimal inflammatory cells in the vitreous. All eyes injected with a 100 or 320 μM dose showed severe retinal destruction and dense inflammatory cell infiltrate in the vitreous, optic nerve, and medullary ray (table 1).

**Treatment of HSV-1 viral retinitis with ISIS 4015.** The natural course and grading system that we used for HSV-1 retinitis in our focal animal model has been described [13, 15] and involves focal retinitis with viral inclusions that expand over a 2-week period to destroy the retina. The eyes were graded using a combination of slit lamp and indirect ophthalmoscopic, histologic, and immunopathologic criteria to accurately determine the presence and extent of retinitis as described above, which resulted in a grading system of 0–4 plus retinitis. Statistical analysis was performed with the Wilcoxon rank sum test using JMP 3.0 (SAS Institute, Carey, NC). We tested for differences in grades of retinitis in treated animals compared with controls (table 2).

The retinitis in control (untreated) animals progressed as follows: 3–4 days after virus inoculation, optic nerve and medullary ray congestion and small splinter-like hemorrhages on the nerve head and throughout the medullary ray were observed. By day 5, small focal patches of retinitis characterized by focal whitening of the retina were seen close to the injection site. Mild vitritis was also seen. By day 7, optic nerve and medullary ray congestion became very severe; the retinitis spread, involving about half of the retina, and the vitreous haze became more severe. By day 10, moderate to severe vitritis was present. The retina, when visible, presented diffuse involvement. By day 14, vitreous hemorrhage and retinal detachments were commonly seen.

**Table 2.** Experimental designs and results of treatment of HSV-1 retinitis.

<table>
<thead>
<tr>
<th>Drug given, final intravitreal concentration</th>
<th>Time drug was given before virus inoculation (days)</th>
<th>No. of rabbits</th>
<th>Grade of viral retinitis (score of each eye)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>15</td>
<td>4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4</td>
<td></td>
</tr>
<tr>
<td>Ganciclovir</td>
<td>571 μM</td>
<td>3</td>
<td>4, 4, 4</td>
<td></td>
</tr>
<tr>
<td>ISIS 4015</td>
<td>32 μM</td>
<td>3</td>
<td>1, 2, 4</td>
<td>.002</td>
</tr>
<tr>
<td></td>
<td>10 μM</td>
<td>7</td>
<td>4, 4, 3.5</td>
<td>.037</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>0, 0, 1.5, 1.5, 2.5, 2.5, 2.5, 2.5</td>
<td>.001</td>
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<td></td>
<td>3</td>
<td>4, 4, 3.5</td>
<td>.037</td>
</tr>
<tr>
<td></td>
<td>3 μM</td>
<td>1</td>
<td>4, 4, 4</td>
<td></td>
</tr>
</tbody>
</table>

NOTE. Results of treatment with ISIS 4015 on focal HSV retinitis in rabbit eyes according to grading system, comparing treated and control animals. Classification was based on worst clinical presentation during course of infection. Grading was on scale of 0 (no retinitis) to 4 (fulminant retinitis). Treated animals with similar but delayed clinical presentation, compared with natural course, had 0.5 deducted from score for statistical purposes (see text).

* Study eyes were compared with those of control animals (n = 15) using Wilcoxon rank sum test.
Figure 1. Photomicrographs of rabbit retina 2 weeks after injection with non–anion exchange–purified SAX™ ISIS 2922 (10 μM final intravitreal concentration). A, Inflammatory cells in vitreous and inflammatory infiltration of medullary ray (arrow) (methacrylate-embedded 1-μm-thick section, toluidine blue–stained ×100). B, Same eye showing shortening and disruption of outer segments and changes on retinal pigment epithelium (methacrylate-embedded 1-μm-thick section, toluidine blue–stained ×250).

with HSV-1 resulted in a markedly attenuated retinitis that had statistically significantly less fulminant grading than that in the control animals (P < .001). Similar experiments done with ISIS 4015 using a 10 μM final intravitreal concentration showed a therapeutic effect 1 day prior to virus inoculation (P = .001; figure 4) and a very weak but statistically significant therapeutic effect when injected 7 days before virus inoculation. To determine the duration of effect of this dose (also the highest nontoxic dose of the compound in the rabbit eye), drug was injected 7 days prior to retinal infection. A mild but statistically significant amelioration of retinitis was seen. Therapy of established retinitis 3 days after virus inoculation was studied. In this scenario, 10 μM ISIS 4015 effected a very weak but still statistically significant amelioration of retinitis in 1 of 3 animals studied. The 3 μM dose of compound had no effect on retinitis when injected 1 day before infection of the retina (figure 5).

Discussion

Retinitis due to infection with viruses of the herpes family is the most common and most visually threatening intraocular
and the emergence of resistant virus strains associated with long-term therapy have limited the effectiveness of these compounds and demonstrated the need for new drugs and treatment strategies [23]. The development of resistance and the toxicity associated with ganciclovir and foscarnet indicate that there is a need for new antiviral compounds to treat HCMV infections.

Viral retinitis can also occur in immunocompetent patients. Acute retinal necrosis (ARN) is a rapidly progressive viral uveitis. Several viral pathogens have been associated with it. Varicella-zoster virus has been the most frequently implicated virus in this disorder. HSV and CMV have also been associated with ARN to varying degrees. Herpes family virus particles have been demonstrated in retinal biopsies of non-AIDS patients with ARN [24–26].

Synthetic oligonucleotides represent a novel alternative to currently available antiviral drugs. Inhibition of viral replication in cell culture by using synthetic oligonucleotides has been reported for several viruses, including human immunodeficiency virus, HSV, influenza virus, Rous sarcoma virus, vesicular stomatitis virus, and papillomavirus [27–29]. Intravitreal administration of phosphorothioate oligonucleotide agents for retinal diseases would allow direct delivery of the compounds to infected tissue. For this reason, we wished to evaluate the toxicity and antiviral activity of this class of compounds in the eye.

ISIS 2922 is complementary to messenger RNA of the HCMV major IE transcriptional unit, which encodes several proteins responsible for regulation of virus gene expression [13, 30–35] and has properties consistent with an antisense mechanism of action [10, 12]. The mean EC50 of ISIS 2922 against HCMV AD169 is 0.37 ± 0.26 μM [10]. Phosphorothioate oligonucleotides such as ISIS 2922 should inhibit replication of HCMV strains that have evolved resistance to currently used antiviral agents during chronic therapy and could be used either alone or in combination with currently approved therapeutics to ameliorate HCMV disease.

Because of the toxicity of systemic intravenous therapy, local intraocular therapy has been used in some selected patients. An intravitreal sustained-release ganciclovir implant that can release ganciclovir at a steady rate for 4–8 months has recently been developed and may prove more effective than the current local treatments [7, 20–22]. However, it involves one or more surgical procedures and complications can occur. Drug toxicity

![Figure 2. Normal retinal appearance in rabbit eye injected with anion exchange–purified SAX+ ISIS 2922 (10 μM final intravitreal concentration (methacrylate-embedded 1-μm-thick section, toluidine blue–stained ×800).](https://academic.oup.com/jid/article-abstract/175/6/1308/1023407)
in humans, the toxicity of the phosphorothioate oligonucleotide compounds was confirmed with high doses. ISIS 2922 injected into 3 pig eyes at two dose levels with two different grades of material suggests that, even with contaminated material, the lack-of-effect dose level is 5 μM. With the 20 μM dose, there was both retinal toxicity and inflammation. Due to the small number of eyes used, these data are not strongly conclusive.

Clinical trials that are ongoing will further evaluate the safety profile and therapeutic index of ISIS 2922 in the vascularized human retina. Both rabbit and pig eyes showed toxicity with intravitreal injections of high doses of ISIS 2922. These models may be useful for developing an understanding of the mechanisms of ISIS 2922. Future research directed at better understanding of the mechanism of ocular inflammation and toxicity following intravitreal administration of ISIS 2922 in the rabbit may prove useful. Our studies suggest that the therapeutic index, from the perspective of in vivo intraocular inflammation, is between 3 and 9 (1 − 3/0.37) and, from the perspective of irreversible retinal toxicity, is between 13 and 54 (5 − 20/0.37). These numbers are based on the highest nontoxic dose divided

Figure 3. Photomicrograph of rabbit retina 2 weeks after injection with ISIS 4015 (320 μM final intravitreal concentration). Retina is severely disorganized (methacrylate-embedded 1-μm-thick section, toluidine blue–stained × 800).

Figure 4. Paraffin section of retina of rabbit treated with ISIS 4015 (10 μM final intravitreal concentration) 1 day before HSV-1 inoculation and sacrificed 21 days after virus inoculation. Immunocytochemistry analysis revealed no evidence of HSV antigen. Retina shows no inflammatory infiltrate and normal cytoarchitecture (counterstained with hematoxylin × 240).
by the dose necessary to inhibit HCMV (mean EC_{50}) [10]. Studies of ISIS 2922 in pigs confirmed toxicity and inflammatory results in a holangiotic retina more similar to that of the human.

Although the therapeutic index is relatively low, only studies in humans can allow more precise determination of the highest tolerated dose. Unfortunately, we only used the ultrapure form of ISIS 2922 (SAX+ ref) at very high concentrations (i.e., 40 \mu M in rabbit eyes, 20 \mu M in pig eyes). The level of impurity of ISIS 2922 (SAX-) and the anion-exchange–purified (SAX+ ref) form make the results of this study far from conclusive regarding ocular toxicity and application in clinical studies due to a new ultrapure compound of ISIS 2922 (SAX+ ref) now available. It is interesting that with both ISIS 2922 (anti-CMV) and ISIS 4015 (anti-HSV) compounds, intraocular inflammation occurred at concentrations lower than those causing irreversible structural changes at the retina. Our studies also suggest that, at least in the rabbit model, purification of these compounds causes a moderate reduction in their proinflammatory and toxic properties. Further studies with uncontaminated SAX-purified material should be performed to allow for the calculation of a therapeutic index for the more clinically relevant dose form. Clearly, even the purest ISIS 2922 was toxic in rabbits at 40 \mu M. It is not possible to draw accurate conclusions as to the actual toxicity of the ultrapure reference standard in the rabbit.

Our studies of therapeutic effects of ISIS 4015 showed a definite antiviral effect in the retinitis model in preventing retinitis (table 2). In some cases, the antiviral activity of oligonucleotides appears to be due to an antisense mechanism of action in which oligonucleotides of defined sequence bind to complementary sequences on target RNA molecules by Watson-Crick base-pairing, resulting in effective and specific inhibition of gene expression [1, 29, 36]. ISIS 4015 was originally designed as an antisense oligonucleotide and it does have potent and specific antiviral activity. Its mechanism of action is sequence-specific but may be related to a repetitive strings of guanine bases, indicating a combination of true antisense and nonantisense effects. Random oligonucleotide sequences did not produce antiviral effects. Inhibition of viral replication by oligonucleotides noncomplementary to viral mRNA has also been reported [28, 36], suggesting that oligonucleotide inhibition of viral replication can involve mechanisms other than antisense.

To test the efficacy of ISIS 4015, we elected to use an animal model of HSV-induced focal progressive retinitis to evaluate the duration of action and efficacy of intravitreal injection. Our model produces a focal, nonlethal, expanding retinitis in the rabbit that spreads in a predictable manner to infect 100% of eyes inoculated. From the perspective of local intravitreal therapy for CMV or HSV retinitis, we used the highest nontoxic dose of the anti-HSV antisense and found that this dose (10 \mu M), when given 1 day prior to viral inoculation, did significantly inhibit retinitis, as did the higher tested dose (32 \mu M) in our treatment studies. Testing of the 10 \mu M dose given 1 week before virus inoculation showed a more modest but definite amelioration of the retinitis. In our HSV-1 retinitis model, we have previously shown that intravitreal ganciclovir (the same lot of compound used in this study) does have a therapeutic effect when given on the day of virus inoculation or early in the course of retinitis [15, 37]. The intravitreal dose we use is equivalent to the final intravitreal concentration in the human eye after a standard 200-\mu g injection [6, 7].

In conclusion, we have demonstrated that there are dose-related ocular toxicities associated with intravitreal injections.
of ISIS 2922 and ISIS 4015, but these occur at concentrations well above therapeutic levels. Additional studies have been conducted to investigate the ocular toxicity of ISIS 2922 in monkey eyes. On the basis of results in primate studies, clinical trials have begun testing ISIS 2922 as an intracocular therapeutic agent in patients with AIDS and CMV retinitis [38].

References


