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Antiviral activity of a phosphorothioate oligonucleotide complementary to human cytomegalovirus RNA when used in combination with antiviral nucleoside analogs

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Abstract

ISIS 2922 is a phosphorothioate oligonucleotide with potent antiviral activity against human cytomegalovirus (HCMV) in cell culture assays. The ability of ISIS 2922 to inhibit replication of HCMV when used in combination with other antiviral agents approved for treatment of HCMV disease was investigated using a 96-well immunoassay. The antiviral activity of ISIS 2922 against HCMV was additive with that of ganciclovir (9-(1,3-dihydroxy-2-propoxymethylguanine); DHPG) or foscarnet (phosphonoformate). Compounds used clinically for the treatment of human immunodeficiency virus infection and likely to be co-administered with ISIS 2922 in the clinic were also evaluated for their ability to modulate the antiviral activity of ISIS 2922. 3′-Azido-3′-deoxythymidine (AZT) exhibited no antiviral activity against HCMV in the 96-well immunoassay, and did not significantly alter the antiviral activity of ISIS 2922. 2′-3′-Dideoxycytidine (ddC) was able to inhibit replication of HCMV at high doses, and this activity was additive with that of ISIS 2922. ISIS 2922 inhibited HIV replication in acute infection assays at relatively high concentrations as previously reported for non-complementary phosphorothioate oligonucleotides. When ISIS 2922 was used in combination with AZT in this assay, interactions were additive at most

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concentrations, although significant and reproducible synergy was observed at some concentration combinations.

Keywords: Antisense oligonucleotide; Human cytomegalovirus; Human immunodeficiency virus; Drug interaction; Combination therapy

1. Introduction

ISIS 2922 is a phosphorothioate oligonucleotide complementary to messenger RNA encoding regulatory proteins of immediate early region 2 of human cytomegalovirus (HCMV). This oligonucleotide exhibits potent, specific antiviral activity against HCMV when evaluated in plaque reduction assays, infectious virus yield reduction assays, and 96-well immunoassays (Azad et al., 1993). One potential therapeutic indication for ISIS 2922 is the treatment of HCMV-induced retinitis in AIDS patients.

In formulating strategies to investigate the potential clinical use of a novel antiviral agent such as ISIS 2922, it is necessary to consider interactions between ISIS 2922 and approved antiviral agents which could be administered simultaneously in the intended patient population. Current treatment for HCMV retinitis involves long-term parenteral administration of the nucleoside analogs ganciclovir (9-(1,3-dihydroxy-2-propoxymenthyl guanine); DHPG) or foscarnet (phosphonoformate). In addition, AIDS patients with HCMV retinitis may also receive therapy for human immunodeficiency virus (HIV) infection with the nucleoside analogs 3′-azido-3′-deoxythymidine (AZT) or 2′,3′-dideoxycytidine (ddC).

To investigate the interactions of ISIS 2922 and antiviral nucleoside analogs, the antiviral activity of ISIS 2922 was evaluated in a 96-well immunoassay for HCMV replication in combination with ganciclovir, foscarnet, AZT and ddC. Potential interactions between ISIS 2922 and AZT were also investigated using an acute infection assay for HIV replication.

2. Materials and methods

2.1. Antiviral compounds

ISIS 2922 was synthesized and purified as previously described (Azad et al., 1993). The sequence of ISIS 2922 is 5′-GCG TTT GCT CTT CTT CTT GCG-3′, which corresponds to nucleotide coordinates 170,120–170,140 on the HCMV AD169 genome (Chee et al., 1990). ISIS 2922 is complementary to sequences present on major immediate early region 2 (IE2) mRNA encoding 55 and 86 kDa polypeptides. Reverse-phase HPLC purified material was used for combination studies with ganciclovir, foscarnet and AZT. Purity of full-length oligonucleotide in this preparation was 92.9% as judged by denaturing polyacrylamide gel electrophoresis (PAGE). Combination studies with ddC used ethanol precipitated ISIS 2922 which was > 70% pure as judged by denaturing PAGE. Both preparations of ISIS 2922 exhibited comparable antiviral

activity. The predominant contaminants in the preparations were oligonucleotides shorter than ISIS 2922 by one residue and monophosphodiester substituted oligonucleotides. DHPG (Syntex, Corp., Palo Alto, CA) was obtained from a prescription pharmacy. AZT, ddC and foscarnet were obtained from Sigma Chemical Co. (St. Louis, MO). All compounds were dissolved in sterile, distilled water for use. To enhance solubility, the pH of the AZT stock solution was increased to 10 by dropwise addition of 1 M ammonium hydroxide. All stock solutions of test compounds were filter sterilized prior to use in cell culture assays.

2.2. 96-Well immunoassay for HCMV replication

Normal human dermal fibroblast (NHDF) cells from foreskin were obtained from Clonetics Corp (San Diego, CA) and propagated at 37°C in a 5% carbon dioxide atmosphere. Fibroblast growth medium (FGM; Clonetics Corp, San Diego, CA) supplemented with 10% fetal bovine serum (FBS) was used for propagation of cells and production of virus stocks. NHDF cells were used between passages 10 and 30.

Human cytomegalovirus strain AD169 was obtained from the American Type Culture Collection (Rockville, MD). Working virus stocks were prepared in monolayer cultures of NHDF cells. Subconfluent cells were infected at a low multiplicity of infection (m.o.i., $\leqslant 0.1$ PFU/cell) and incubated until uniform cytopathology was observed (7–9 days). After mild agitation, culture supernatant and infected cells were harvested and frozen in 1-ml aliquots at −150°C. Titers of virus stocks were between 1 and 3×10^7 PFU/ml.

Antiviral assays were performed essentially as described previously (Azad et al., 1993). NHDF cells were seeded in 96-well culture plates at a density of 15,000 cells per well in FGM containing 0.2% FBS (FGM–0.2% FBS). Established monolayers were pretreated with test compound(s) overnight in FGM–0.2% FBS prior to infection. After pretreatment, cells were rinsed 3 × in fresh prewarmed FGM–0.2% FBS, and virus in 100 μl FGM–0.2% FBS per well was added to achieve an m.o.i. of 0.05 PFU/cell. After 2 h incubation at 37°C, virus was removed and fresh media (100 μl/well) containing test compound(s) was added. Three days after infection, cells were fixed in absolute ethanol and dried in preparation for antibody staining. Infection times and m.o.i. were chosen to optimize reproducibility of assays and minimize manipulations. The antiviral activity of ISIS 2922 and ganciclovir were not significantly altered using a 6-day incubation prior to fixing (Azad et al., 1993).

Fixed cells were blocked in a solution of phosphate buffered saline (PBS) containing 2% bovine serum albumin (BSA), and mouse monoclonal antibody (1H10 supplied by Eisai Co., Ltd., Japan) was added using a 1 : 2000 dilution in PBS–1% BSA. The 1H10 antibody recognizes an abundant late HCMV polypeptide approximately 65 kDa in size (K. Tanaka, unpublished observation). Detection of bound monoclonal was facilitated using biotinylated goat anti-mouse IgG and streptavidin-coupled ß-galactosidase (Gibco/BRL, Gaithersburg, MD). Chlorophenol red ß-D-galactopyranoside (CPRG) was used as a substrate for ß-galactosidase, and activity was determined by measuring optical density of individual wells at 575 nm using a BioTex model EL312e microplate reader. Each 96-well plate contained a 6 × 6 matrix of drug combinations at different dilutions,

with 12 wells of positive controls (virus infected, untreated cells) and 12 wells of negative controls (uninfected cells). Each assay was performed using triplicate plates, with the results reported as the means of the 3 determinations. Percent antigen expression relative to untreated control cells was calculated as 100 times the difference between optical densities of untreated HCMV-infected cells and test cells, divided by the difference between the optical densities of untreated HCMV-infected cells and uninfected cells. The MacSynergy v5.1 software used to evaluate drug interactions for this study compares values for percent inhibition of virus replication. Percent virus inhibition was defined as 100 minus the percent antigen expression relative to untreated control wells (as defined above). All results in this report are presented as percent virus inhibition.

2.3. Acute infection assay for replication of human immunodeficiency virus

The antiviral activity of ISIS 2922 in combination with AZT against HIV was evaluated using an acute infection assay (Buckheit et al., 1993). The CEM-SS cell line (Nara and Fischinger, 1988) was maintained in RPMI 1640 medium supplemented with 10% FBS, 2 mM glutamine, 100 μg/ml penicillin, and 100 μg/ml streptomycin. HIV-1 strain IIIB was obtained from the NIAID AIDS Research and Reference Reagent Program.

Cells were placed in each well of a 96-well microtiter plate at a density of 5000 cells per well and infected at an m.o.i. previously determined to result in complete cell killing at 6 days postinfection (m.o.i. = 0.01–0.05). Combinations of test-compounds in serial 2-fold dilutions were added to wells and after incubation for 6 days at 37°C, cell viability was determined spectrophotometrically as described by Weislow et al. (1989). XTT-tetrazolium was added to each well, the plates were incubated for 4 h at 37°C and optical density at 540 nm determined. Controls for each plate included drug colorimetric control wells (drug only), drug cytotoxicity (cells plus drug), virus control wells (cells plus virus), and cell viability control wells (cells only). Assays were performed using duplicate plates and results are the means of triplicate determinations for each plate.

2.4. Statistical analysis of drug interactions

Statistical evaluation of drug interactions was performed using a program developed by Prichard and Shipman (1990) to distinguish additive, synergistic, and antagonistic effects (MacSynergy v5.1). This analysis compares observed activities for 2 compounds used in a matrix of drug combination concentrations to calculated theoretical additive activities. If the lower 95% confidence limit of the experimental data for a given cell in the concentration matrix (2 standard deviations) is greater than the theoretical additive activity, then synergistic interactions are considered to be significant for that cell. If the upper 95% confidence limit of the experimental data is less than the calculated additive activity, then antagonistic interaction is considered to be significant. For analysis of interactions between ISIS 2922 and the 4 other compounds investigated (ganciclovir, foscarnet, ddC, and AZT) in the HCMV 96-well immunoassay a dissimilar mechanism of action was assumed. Because of the uncertainty of the mechanism of antiviral activity

of ISIS 2922 in the HIV antiviral assay a similar mechanism of action was assumed. This analysis is more stringent than that employed for evaluating compounds with dissimilar mechanisms.

The 95% confidence limits used for this analysis will result in sporadic statistical anomalies with a frequency of approximately 1 per 20 matrix cells evaluated. Therefore, conclusive demonstration of meaningful synergy or antagonism by this type of analysis included reproducible demonstration of statistical significance over an extended portion of the concentration matrix.

3. Results

3.1. Inhibition of HCMV replication by combination treatment with ISIS 2922 and inhibitors of viral DNA polymerase activity

Four different inhibitors of viral DNA polymerase activity used for the treatment of HCMV or HIV infection (ganciclovir, foscarnet, ddC, and AZT) were each evaluated in combination with ISIS 2922 for antiviral activity against cytomegalovirus. Effects of combination treatment with ISIS 2922 and each of 4 antiviral drugs were evaluated in a 96-well immunoassay for HCMV replication using a 6×6 matrix array of combinations of drug concentrations. Triplicate determinations were evaluated for statistically significant deviations from predicted additive activity using the approach described by Prichard and Shipman (1990). In the case of all 4 antiviral drugs, deviations from predicted additive activity were modest. Differences exceeding the calculated 95% confidence intervals were generally of low magnitude and were not observed over extended portions of the concentration matrix. These isolated statistically significant differences were therefore not considered meaningful indications of synergistic or antagonistic interactions.

Evaluation of the effects of combination treatment with ganciclovir and ISIS 2922 were evaluated in the 96-well immunoassay using half-log dilutions of ISIS 2922 at concentrations of 0.01–1.0 μM and ganciclovir at concentrations of 0.3–30 μM. Fifty percent effective antiviral concentrations (EC_{50}s) for ISIS 2922 and ganciclovir were calculated to be 0.5 and 2 μM, respectively. At most concentrations of ISIS 2922 evaluated, antiviral activity was enhanced in the presence of ganciclovir proportionately to the activity of ganciclovir when used alone at that concentration. Statistically significant deviations indicating potential synergy were observed at 4 different combinations of ISIS 2922 and ganciclovir, but the magnitudes of these deviations were relatively small and a trend indicating synergy over a broad concentration range was not evident (Table 1). Only a single cell in the concentration matrix (10 μM ganciclovir, 0.1 μM ISIS 2922) indicated statistically significant antagonism.

The effects of combination treatment with ISIS 2922 and foscarnet on HCMV replication were evaluated in the 96-well immunoassay using half-log dilutions of ISIS 2922 at concentrations of 0.03–3 μM and foscarnet at concentrations of 3–300 μM. EC_{50}s for ISIS 2922 and foscarnet were determined to be 0.1 and 40 μM, respectively. At most concentrations of ISIS 2922 evaluated, antiviral activity was enhanced in the

Table 1
Differences from predicted additive inhibition of HCMV replication using combination treatment with ISIS 2922 and ganciclovir

Ganciclovir conc. (μM)	ISIS 2922 concentration (μM)				
	0.01	0.03	0.1	0.3	1.0
30	16.1	8.0	13.2 [a]	10.9 [a]	9.3
10	17.1	11.6	11.3 [a]	13.7	10.0
3	−3.1	−4.2	−10.7 [a]	0.6	8.2
1	−3.6	0	2.9	14.7	12.7
0.3	16.0	4.4 [a]	−6.4	0.7	4.2

[a] Differences exceeded 95% confidence interval limits for statistical significance.

presence of foscarnet proportionately to the activity of foscarnet when used alone at that concentration (Table 2). Only a single cell in the concentration matrix (30 μM foscarnet, 1.0 μM ISIS 2922) indicated statistically significant synergism. Similarly, only a single cell in the concentration matrix (300 μM foscarnet, 0.3 μM ISIS 2922) indicated statistically significant antagonism.

The effects of combination treatment with ISIS 2922 and ddC on HCMV replication were also evaluated in the 96-well immunoassay using half-log dilutions of ISIS 2922 at concentrations of 0.03–3 μM and ddC at concentrations of 3–300 μM. Although ddC is not used for treatment of HCMV infection, some inhibition of HCMV replication by ddC alone was observed at higher concentrations. The EC_{50} for ddC inhibition of HCMV replication was calculated to be 300 μM. The EC_{50} for ISIS 2922 inhibition of HCMV replication in this assay was 0.3 μM. When the activities of combinations of ISIS 2922 and ddC were compared to calculated theoretical activities, only modest deviations were observed. In general, at all concentrations of ISIS 2922 evaluated, antiviral activity was enhanced in the presence of ddC proportionately to the activity of ddC when used alone at that concentration. Six cells in the concentration matrix indicated statistically significant synergism, including concentrations of ddC between 10 and 300 μM used in combination with 1 μM ISIS 2922, but the magnitude of the differences relative to predicted additive activity was low (Table 3). Synergy at the next higher dose of ISIS 2922 (3 μM) could not be evaluated since at this concentration of

Table 2
Differences from predicted additive inhibition of HCMV replication using combination treatment with ISIS 2922 and foscarnet

Foscarnet conc. (μM)	ISIS 2922 concentration (μM)				
	0.03	0.1	0.3	1.0	3.0
300	1.6	2.2	−4.1 [a]	−0.3	1.2
100	2.8	4.9	−1.5	0.3	0.8
30	3.9	8.6	−9.3	0.6 [a]	2.3
10	1.2	4.5	−6.6	−0.2	0.7
3	−7.8	1.4	−4.1	−2.1	−0.3

[a] Differences exceeded 95% confidence interval limits for statistical significance.

Table 3
Differences from predicted additive inhibition of HCMV replication using combination treatment with ISIS 2922 and 2′,3′-dideoxycytidine (ddC)

ddC conc. (μM)	ISIS 2922 concentration (μM)				
	0.03	0.1	0.3	1.0	3.0
300	8.6	10.8	8.4	13.4 [a]	4.6
100	−0.9	−2.1	−0.7	14.4 [a]	−3.8 [a]
30	−0.5	0.4	7.2 [a]	15.9 [a]	−1.3
10	9.2	4.5 [a]	5.1	19.0 [a]	−2.4
3	2.1	3.7	9.1	3.1	0.3

[a] Differences exceeded 95% confidence interval limits for statistical significance.

ISIS 2922 HCMV replication is completely inhibited in the absence of ddC. Only a single cell in the concentration matrix (100 μM ddC, 3.0 μM ISIS 2922) indicated statistically significant antagonism.

The effects of combination treatment with ISIS 2922 and AZT on HCMV replication were evaluated in the 96-well immunoassay using half-log dilutions of ISIS 2922 at concentrations of 0.03–3 μM and AZT at concentrations of 3–300 μM. Treatment with AZT alone had no effect on replication of HCMV in this assay. The EC_{50} for ISIS 2922 inhibition of HCMV replication in this assay was 0.5 μM. The concentration-dependent inhibition of HCMV replication by ISIS 2922 was not altered in the presence of AZT in any meaningful manner (Table 4). Statistical comparisons showed 2 cells in the concentration matrix (100 μM AZT, 0.03 μM ISIS 2922 and 10 μM AZT, 1 μM ISIS 2922) with statistically significant deviations indicating potential synergistic interactions, but the magnitudes of those differences were low. No statistically significant deviations indicating antagonism were observed.

3.2. Inhibition of HIV replication by combination treatment with ISIS 2922 and AZT

The effects of combination treatment with ISIS 2922 and AZT on HIV replication were evaluated in an acute infection assay measuring cytopathic effects of viral infection as an endpoint. Duplicate experiments were performed to evaluate combination antiviral

Table 4
Differences from predicted additive inhibition of HCMV replication using combination treatment with ISIS 2922 and 3′-azido-3′-deoxythymidine (AZT)

AZT conc. (μM)	ISIS 2922 concentration (μM)				
	0.03	0.1	0.3	1.0	3.0
300	0.2	16.8	23.4	−0.3	−0.4
100	26.6 [a]	−0.5	2.0	−2.7	0.4
30	−9.6	−5.2	4.2	1.5	−3.3
10	−3.7	−16.1	18.5	9.3 [a]	0
3	−8.2	−8.6	1.9	−1.7	−1.6

[a] Differences exceeded 95% confidence interval limits for statistical significance.

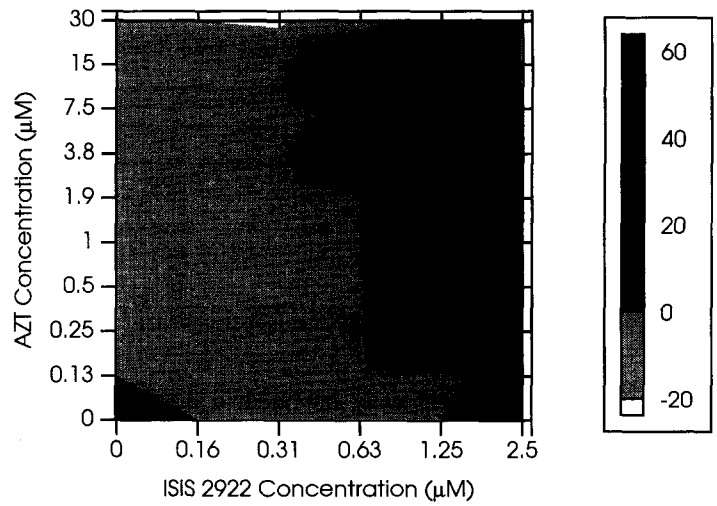

Fig. 1. Differences from predicted inhibition of HIV replication by combination treatment with ISIS 2922 and AZT. The activities of ISIS 2922 and AZT were evaluated for antiviral activity using the acute infection assay for HIV-1 replication described in Materials and methods. Mean differences from predicted inhibition in two experiments using combinations of drug concentrations shown on the x and y axes are represented as contours of a three-dimensional surface plot.

activity. In both experiments, CEM-SS cells infected with HIV-1 were treated with 2-fold dilutions of ISIS 2922 between 0.16 and 2.5 μM in combination with 2-fold dilutions of AZT between 0.125 and 30 nM.

ISIS 2922 treatment alone inhibited HIV replication in both assays with identical EC_{50} values of 2 μM. Calculated EC_{50} values for AZT inhibition of HIV replication

Table 5
Differences from predicted additive inhibition of HIV replication using combination treatment with ISIS 2922 and 3′-azido-3′-deoxythymidine (AZT)

AZT conc. (μM)	ISIS 2922 concentration (μM)				
	0.16	0.31	0.63	1.25	2.5
30	−20.5 [a,b]	−24.1	−10.7	24.3 [b]	−3.5
15	−4.1	−1.6	28.6	53.0 [b]	−3.5
7.5	−2.3	−4.4	2.2	55.8 [b]	−0.2
3.8	−0.7	−1.1	7.8	55.6 [b]	5.3
1.9	−2.4	−3.3	−1.1	37.3	6.4
1.0	−2.8	−2.7	−2.3	22.1	2.0
0.5	−1.6	−2.0	−1.4	18.2	8.7
0.25	−1.3	−2.1 [b]	−1.9	21.8	9.7
0.125	−5.0 [b]	−3.7 [b]	−3.9 [b]	−2.5	6.3

[a] Numeric values are the means of 2 experiments. Each experiment was performed using 3 replicate wells for each drug combination.
[b] Differences exceeded 95% confidence interval limits for statistical significance in both experiments.

were 26 nM (7 ng/ml) in both assays. When antiviral activities of combinations of ISIS 2922 and AZT were compared to calculated theoretical additive activities, significant synergy was observed for several drug combinations (Fig. 1, Table 5). Reproducible synergy was observed in both assays using AZT concentrations between 3.8 and 15 μM in combination with 1.25 μM ISIS 2922. Differences exceeded predicted additive activity by 50 to 61% and exceeded the 95% confidence intervals for predicted additive activity by 25 to 52%. The magnitude of the differences relative to predicted additive activities, and the pattern of synergy in several adjacent cells of the concentration matrix in two separate assays provide convincing evidence of synergistic interactions at these concentrations. Statistically significant negative deviations from predicted additive activities which would indicate antagonistic interactions were outside 95% confidence limits by only relatively small amounts ($< 4\%$) or were not reproducible in both assays. Uninfected cells were treated and evaluated in parallel with infected cells in this assay as a measure of cytotoxicity. No evidence of cytotoxicity was observed in uninfected cells at any of the concentration combinations evaluated in this assay.

4. Discussion

ISIS 2922 was evaluated for its ability to inhibit HCMV replication in combination with ganciclovir and foscarnet, two licensed drugs used for the treatment of HCMV retinitis. ISIS 2922 inhibited HCMV replication in an additive manner when used in combination with either ganciclovir or foscarnet over broad matrices of concentration combinations. While a few combinations of compounds at specific concentrations showed differences which were nominally significant relative to predicted additive activities, the magnitude of the differences was relatively small and significant differences were not observed over any extended range of concentrations. Using a 95% confidence interval for evaluation of significance, such anomalies would be predicted to occur in approximately 1 of each 20 comparisons. Therefore, these differences are likely due to statistical variation. The data demonstrate that combinations of foscarnet or ganciclovir with ISIS 2922 can inhibit HCMV replication in cell culture in an additive manner.

As HCMV retinitis is primarily a disease of late stage AIDS patients, it is likely that a large proportion of patients requiring therapy for HCMV retinitis will also be receiving therapy for HIV infection. AZT and ddC are nucleoside analogs which inhibit HIV replication in cell culture and which are used clinically for the treatment of HIV infection. Since these antiviral compounds are likely to be co-administered with ISIS 2922 in clinical trials, the effects of these compounds on the anti-HCMV activity of ISIS 2922 were investigated.

Dideoxycytidine, although not reported to have activity against HCMV, showed some inhibition of HCMV replication at high concentrations in our assays. This inhibition appeared to work additively in combination with ISIS 2922 at most combinations evaluated. Some evidence of synergistic interaction was observed at concentrations of ddC between 10 and 300 μM when used with ISIS 2922 at 1 μM. However, differences exceeding a 95% confidence interval were small in magnitude and did not provide

conclusive evidence of synergistic interaction. Antagonism of antiviral activity using ISIS 2922 and ddC in combination was not observed.

AZT showed no antiviral activity against HCMV at the concentrations tested, and no statistically significant antagonistic interactions were observed when used in combination with ISIS 2922. Statistically significant enhancement of the antiviral activity of ISIS 2922 in the presence of AZT was noted in only two isolated cells of the concentration matrix and were likely due to experimental variability. It is unlikely that concurrent therapy using ddC or AZT in patients treated with ISIS 2922 will adversely affect the anti-HCMV activity of ISIS 2922.

Potential interactions between ISIS 2922 and AZT were also investigated in an acute infection assay for HIV replication. In two separate assays ISIS 2922 treatment alone inhibited HIV replication at relatively high concentrations ($EC_{50} = 2$ μM). This inhibition is probably similar in nature to antiviral activity described for other non-complementary phosphorothioate oligonucleotides on HIV-1 replication (Matsukura et al., 1987). Reproducible synergism between ISIS 2922 and AZT in this assay was observed. AZT concentrations of 3.8–15 nM (1–4 ng/ml) in combination with 1.25 μM ISIS 2922 showed statistically significant enhancement of predicted additive activities. The magnitude of the differences relative to predicted additive activities, and the pattern of synergy in several adjacent cells of the concentration matrix in two separate assays provide convincing evidence of synergistic inhibition of HIV replication at these concentrations. No meaningful pattern of antagonism between ISIS 2922 and AZT in the HIV antiviral assay was noted. The apparent synergistic interactions between AZT and ISIS 2922 should be considered in the design and evaluation of clinical trials utilizing systemic administration of phosphorothioate oligonucleotides in AIDS patients receiving concurrent AZT therapy.

ISIS 2922 exhibited positive interactions in antiviral assays with all 4 antiviral agents evaluated (ganciclovir, foscarnet, AZT, and ddC). ISIS 2922 exhibited additive antiviral activity against HCMV when used in combination with ganciclovir or foscarnet, two drugs administered clinically for the treatment of HCMV retinitis in AIDS patients, and the ability of ISIS 2922 to inhibit HCMV replication in a cell culture assay was not adversely affected when used in combination with ddC or AZT, two drugs likely to be administered as anti-HIV therapy in AIDS patients with HCMV retinitis. Furthermore, ISIS 2922 demonstrated non-antisense inhibition of HIV-1 replication at relatively high concentrations which was additive when used in combination with AZT at most concentration combinations, but which exhibited significant and reproducible synergy with AZT at some concentration combinations. The positive interactions between ISIS 2922 and antiviral agents currently used in the clinic provide experimental support for the evaluation of combination therapies using ISIS 2922 and nucleoside analogs in human clinical trials.

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