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2-Acetylpyridine 5-[(Dimethylamino)Thiocarbonyl]-Thiocarbonohydrazone (1110U81) Potently Inhibits Human Cytomegalovirus Replication and Potentiates the Antiviral Effects of Ganciclovir

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We studied the effects of 2-acetylpyridine 5-[(dimethylamino)thiocarbonyl]-thiocarbonohydrazone (1110U81 or A1110U), a potent inhibitor of the ribonucleotide reductases encoded by herpes simplex virus types 1 and 2 and by varicella-zoster virus, against human cytomegalovirus (HCMV) replication in infected MRC-5 cells. We show that 1110U81 is a potent inhibitor of HCMV DNA replication (50% inhibitory concentration [IC_{50}], 3.6 μM; IC_{90}, 5.6 μM) and also potentiates the effects of ganciclovir (GCV) against HCMV. The IC_{90} of GCV is reduced from 65 μM when GCV alone is given to 2.8 μM when GCV is combined with 1110U81 at a molar ratio of 1:1.

Ribonucleotide reductase is a key enzyme in the biosynthesis of deoxynucleotides in eukaryotic and prokaryotic organisms (22). This enzyme catalyzes the rate-limiting step in the de novo synthesis of the deoxyribonucleotides from ribonucleotides (13). Several members of the herpesvirus family, namely, herpes simplex virus type 1 (HSV-1), HSV-2 (1, 2, 7, 19), varicella-zoster virus (VZV) (19), and Epstein-Barr virus (9), have been shown to encode their own ribonucleotide reductase enzymes. The viral enzymes are biochemically distinct from the isofunctional counterparts (16, 17), and therefore, the viral enzymes may serve as potential targets for antiviral drugs.

Spector et al. (18–21) have described a new group of antiherpesvirus agents that are potent inhibitors of the ribonucleotide reductases of HSV-1, HSV-2, and VZV. 2-Acetylpyridine 5-[(dimethylamino)thiocarbonyl]-thiocarbonohydrazone (1110U81 or A1110U) inhibits the replication of HSV-1 and potentiates the activity of acyclovir against HSV-1 (19). In an extension of the work done by Spector et al. (19), our study defines the activity of 1110U81 against human cytomegalovirus (HCMV) DNA replication and the potentiation of anti-HCMV effects of ganciclovir (GCV) by 1110U81.

GCV and 1110U81 were obtained from the Burroughs Wellcome Co., Research Triangle Park, N.C.; GCV is now available from Syntex, Palo Alto, Calif. 1110U81 was prepared fresh in distilled water on the day of each experiment. Human embryonic lung fibroblasts (MRC-5 cells) were obtained from the American Type Culture Collection (Rockville, Md.). MRC-5 cells and HCMV (Towne strain) were maintained and passaged, and the confluent cells were infected at 1 to 3 PFU/cell, as previously described (12). The HCMV-infected cells were collected by trypsinization 4 days after infection. The cells were stored at −80°C until dot blot preparation.

HCMV DNA replication was measured by dot blot DNA-DNA hybridization with a cloned *Bam*HI-Q fragment from the end of the HCMV genome as a probe (12). Total cellular and viral DNA was processed for dot blot DNA-DNA hybridization, as previously described (11, 12). The *Bam*HI-Q fragment (11, 12) from HCMV (Towne) was prepared as described by LaFemina and Hayward (15), Thomsen and Stinski (23), and D'Aquila et al. (6). The cloned fragment probe was a gift from G. Hayward, The Johns Hopkins University. Prehybridization, hybridization, washing of the dot blots, and determination of the radioactivity associated with the probe were performed as previously described (12).

Toxicity of GCV and 1110U81 was tested individually and in combination in uninfected MRC-5 cells on the same day as the antiviral activity experiments. The ranges of GCV concentrations tested were 0.1 to 128 μM when GCV was used alone, 0.1 to 16 μM in 1:0.5 combination experiments, and 0.1 to 8 μM in 1:1 combination experiments. The range of 1110U81 concentrations tested was 0.1 to 12.8 μM when 1110U81 was used alone.

Anti-HCMV activity of 1110U81 and potentiation of GCV. 1110U81 inhibited HCMV DNA replication in a concentration-dependent manner (Fig. 1A). The 50% inhibitory concentration (IC_{50}) and IC_{90} of 1110U81 were 3.6 and 5.9 μM, respectively (Table 1). GCV also inhibited HCMV DNA replication with an IC_{50} and IC_{90} of 5.6 and 64 μM, respectively (Table 1).

Combinations of GCV and 1110U81 at fixed ratios (1:0.5 and 1:1 [GCV/1110U81]) (Fig. 1B) resulted in a shift to the left of the dose-response curves, especially at higher concentrations of GCV. The IC_{50}s of GCV were reduced by 59 and 73% and the IC_{90}s were 92 and 96% when the drugs were combined at 1:0.5 and 1:1 ratios (GCV/1110U81) compared with those for GCV alone (Table 1).

GCV and 1110U81 were synergistic by both the isobologram method (3) and the median effect method (5). By using the combination index (CI) with either an exclusive or nonexclusive (more conservative) analysis, 1110U81 and GCV were synergistic. Depending on the ratio studied (5), CI_{50}s ranged from 0.69 to 0.85 and CI_{90}s ranged from 0.57 to 0.61 (a combination index of less than 1 suggests synergy).

We studied the toxicity of GCV, 1110U81, and combina-

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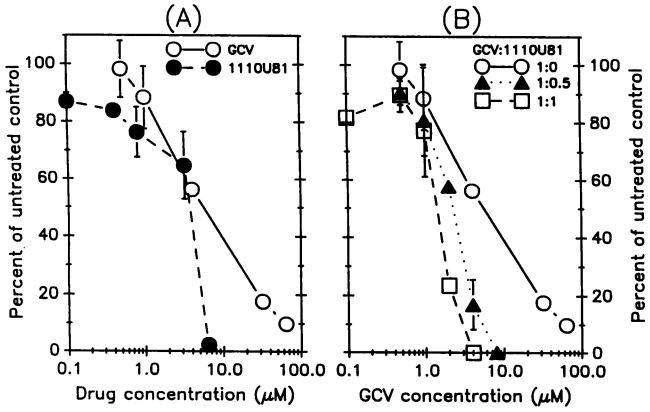

FIG. 1. Dose-dependent inhibition of HCMV DNA replication by GCV and 1110U81 separately (A) and combined (B) at fixed molar ratios of 1:0.5 and 1:1 (GCV/1110U81). HCMV DNA was measured by dot blot DNA DNA hybridization with a cloned HCMV DNA probe. This experiment is representative of four experiments. Each datum point represents the mean and standard deviation of triplicate determinations.

tions of the two drugs to nonconfluent (dividing) and confluent (nondividing) uninfected MRC-5 cells. The drugs were added to nonconfluent uninfected cells 24 h after plating. The cells were incubated with the drugs for 4 days (3 to 4 doubling times), which is the incubation time of infected cells in the efficacy studies. Another set of cultures was allowed to grow to confluence before addition of the drug for 4 days. Toxicity, as measured by the number of viable cells in treated compared with untreated control cultures, was predominantly due to the toxicity of 1110U81 by itself. The highest concentration of GCV (128 μM) alone reduced the numbers of viable cells by 5% in confluent cultures and by 10% in nonconfluent cultures compared with that of untreated controls. The highest concentration of 1110U81 (12.8 μM) alone reduced the numbers of viable cells by 49% in confluent cultures and by 57% in nonconfluent cultures compared with that of untreated controls. The combination of GCV (16 μM) and 1110U81 (8 μM) (1:0.5 ratio) reduced the numbers of viable cells by 36% in confluent cultures and by 51% in uninfected nonconfluent cultures; the same combination produced almost complete inhibition of viral DNA replication in infected cells (Fig. 1B). The combination of 8 μM GCV and 8 μM 1110U81 (1:1 ratio) reduced the numbers of viable cells by 41% in confluent cultures and by 51% in nonconfluent cultures, while the combination of 4 μM GCV with 4 μM 1110U81 produced almost complete inhibition of viral DNA replication (Fig. 1B). Thus, 1110U81 had low selectivity to MRC-5 cells, but its cellular toxicity was not potentiated by GCV. Previous studies demonstrated that acyclovir does not affect the toxicity of 1110U81 (19). We also tested the effects of 1110U81 on HSV DNA replication in our culture system (data not shown), using dot blot DNA-DNA hybridization, and found it to be consistent with a previous report by Spector et al. (19).

TABLE 1. Effects of GCV alone and combined with 1110U81 on HCMV DNA replication[a]

GCV-1110U81 drug combination	GCV effect on HCMV[b]	
	IC_{50} (μM)	IC_{90} (μM)
1:0	5.6 ± 0.32	64.6 ± 5.4
1:0.5	2.3 ± 0.18	5.3 ± 0.56
1:1	1.5 ± 0.1	2.8 ± 0.23

[a] The IC_{50} for 1110U81 alone is 3.6 ± 0.45 μM, and the IC_{90} for 1110U81 alone is 5.9 ± 0.26 μM.

[b] Data are the means and standard deviations from three experiments.

We demonstrated that 1110U81 is a potent inhibitor of HCMV DNA replication and potentiated the anti-HCMV activity of GCV. 1110U81 is a potent inhibitor of the HSV and VZV ribonucleotide reductases (18, 19). 1110U81 was also found to be a weaker inhibitor of the cellular ribonucleotide reductase (19). The selectivity of 1110U81 toward the viral enzymes, as described by Spector et al. (19), was adequate to allow the demonstration of synergistic potentiation of the activity of acyclovir against HSV-1 and VZV replication (8, 19). These viruses are known to code for their own ribonucleotide reductases (1, 7). We confirmed that 1110U81 is as potent against HCMV as it is against HSV-1 (data not shown). Furthermore, because 1110U81 potentiated GCV's anti-HCMV activity and acyclovir's anti-HSV activity (19 and data not shown) with comparable efficiency, the mechanism by which 1110U81 inhibits HCMV DNA replication may be analogous to the suggested mechanism of inhibition of HSV and VZV DNA replication.

The antiviral activities of 1110U81 and similar ribonucleotide reductase inhibitors (A723U and 348U87) (14, 20) are thought to be due to the selective inhibition of the virus-encoded ribonucleotide reductase. However, it is also possible that the activity of 1110U81 against HSV and VZV is partially due to the inhibition of the cellular ribonucleotide reductase. The possibility that 1110U81 is inhibiting the cellular enzyme is supported by the failure to show, by sequence homology studies or enzyme purification studies, that HCMV encodes an active ribonucleotide reductase. Furthermore, an HSV-1 mutant lacking the large subunit of ribonucleotide reductase (10) (which is thought to be essential for enzymatic activity) is able to grow in tissue culture, suggesting that the inhibition of the viral ribonucleotide reductase alone is not sufficient to explain the degree of viral DNA synthesis inhibition described by Spector et al. (19) or seen in our experiments. In either case, whether the viral or cellular ribonucleotide reductase is inhibited, the data suggest that HCMV DNA synthesis is preferentially inhibited compared with the inhibition of cellular DNA synthesis. The inhibition of ribonucleotide reductase may be deleterious only to the rapidly replicating viral DNA and not to the nonreplicating cellular DNA. HCMV infection is characterized by severalfold increases in the intracellular deoxynucleotides (4), which may be required for efficient viral DNA synthesis. Inhibition of this buildup of deoxynucleotide pools by 1110U81 may explain the preferential inhibition of viral DNA synthesis and may also explain why GCV and 1110U81 are synergistic only against HCMV DNA replication and not synergistic against cellular DNA replication in uninfected nonconfluent (dividing) and confluent (nondividing) cells.

In summary, 1110U81 was a potent inhibitor of HCMV DNA replication at concentrations comparable to those that inhibit HSV DNA replication. 1110U81 potentiated the effects of GCV against HCMV, as well as the effects of ACV against HSV. This inhibition of HCMV replication by 1110U81 and the synergistic interaction of 1110U81 and GCV may be due to inhibition of an unrecognized HCMV-encoded ribonucleotide reductase or could result from HCMV being sensitive to the inhibition of the cellular ribonucleotide reductase.

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