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Anti-Human Immunodeficiency Virus Type 1 Activity of Hydroxyurea in Combination with 2′,3′-Dideoxynucleosides

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SUMMARY

The effects of hydroxyurea (HU), an inhibitor of ribonucleotide reductase, on the replication of human immunodeficiency virus type 1 (HIV-1) in activated peripheral blood mononuclear cells were studied. The inhibition of HIV-1 replication by HU alone was dose dependent, with a 90% inhibitory concentration of 0.4 mM, a plasma concentration tolerated by patients with oncological diseases. HU at lower concentrations (<0.1 mM) was found to potentiate the antiviral activity of 2′,3′-dideoxyinosine (ddI), 3′-azido-2′,3′-dideoxythymidine, and 2′,3′-dideoxycytidine against HIV-1, with the potentiation being ddI greater than 3′-azido-2′,3′-dideoxythymidine = 2′,3′-dideoxycytidine. In the presence of 0.1 mM HU, the 90% inhibitory concentration of ddI was reduced by 6-fold in activated peripheral blood mononuclear cells. The potentiating effect of HU on ddI action was time dependent, with the greatest inhibition of HIV-1 growth being seen when HU was present during and after virus adsorption, i.e., apparently coinciding with the time of proviral DNA synthesis. A brief incubation of activated cells with HU and ddI at low concentrations before virus exposure reduced p24 production by >50%. Analyses using high performance liquid chromatography and enzymatic assays suggested that the greater degree of potentiation by HU of the action of ddI, compared with the other dideoxynucleosides, is due to the more effective inhibition by HU of dATP synthesis, compared with the synthesis of the other deoxynucleoside triphosphates (dGTP, dTTP, and dCTP). The present study suggests that, for appropriate agents, pharmacological reduction of deoxynucleoside triphosphate levels represents a potential therapeutic approach for inhibition of HIV-1 replication.

The conversion of the four ribonucleotides to deoxyribonucleotides is a rate-limiting step for DNA synthesis and is catalyzed by ribonucleotide reductase (1–4). This enzyme is a primary target of HU action, and the site of inhibition has been localized to the smaller of the two enzyme subunits (5–7). HU is reportedly able to quench the tyrosyl free radical at the active site of the small subunit and inactivates the enzyme (7). As a result, cellular DNA synthesis is selectively inhibited. Although the detailed mechanism of HU action is still a subject of investigation, this compound has been approved in the United States for the treatment of oncological diseases since 1960. HU is well absorbed, enters cells by passive diffusion, distributes throughout body water, including the cerebrospinal fluid, and is excreted in the urine with a biological half-life of <8 hr (8, 9). Because HU inhibits DNA synthesis and has a brief duration, the drug is toxic only to cells that are actively synthesizing DNA during the period of drug exposure. Bone marrow suppression appears to be a potential dose-limiting toxicity (10). Other toxicity in long term daily maintenance therapy includes partial alopecia, increased pigmentation, and atrophy of the skin (11). The clinical trials over the past 30 years have shown that such HU toxicities can be reversed using schedules of intermittent treatment (8, 11–14).

Inasmuch as HU can selectively inhibit DNA synthesis of malignant cells by depleting the dNTP precursors, it may also inhibit HIV-1 proviral DNA synthesis in infected cells, because the rate of HIV-1 replication is significantly greater than that of host cell growth. This hypothesis was recently explored by Gao *et al.* (15). The results indicated that HU at 1 mM decreased intracellular dNTP pools to the levels seen in quiescent PBM and thus inhibited HIV-1 proviral DNA synthesis in activated PBM. dATP synthesis was inhibited more rapidly and profoundly than that of the other dNTPs, a result that has been previously noted in other systems by Bianchi *et al.* (16) and by Slabaugh *et al.* (17).

Furthermore, because dideoxynucleosides (as their pharmacologically active nucleotides [ddNTPs, 2′,3′-dideoxynucleoside 5′-triphosphates]) compete with endogenous dNTPs for inhibition of HIV-1 reverse transcriptase and for incorporation into growing proviral DNA, we felt it possible that depleting dNTP pools with HU (or with other ribonucleotide reductase inhibitors) could potentiate the activity of agents such as ddI

ABBREVIATIONS: HU, hydroxyurea; PBM, peripheral blood mononuclear cells; ddI, 2′,3′-dideoxyinosine; IC_{90}, 90% inhibitory concentration; dNTP, deoxynucleoside 5′-triphosphate; AZT, 3′-azido-2′,3′-dideoxythymidine; ddC, 2′,3′-dideoxycytidine; HIV, human immunodeficiency virus; PHA, phytohemagglutinin; AIDS, acquired immunodeficiency syndrome; ddATP, 2′,3′-dideoxyadenosine 5′-triphosphate.

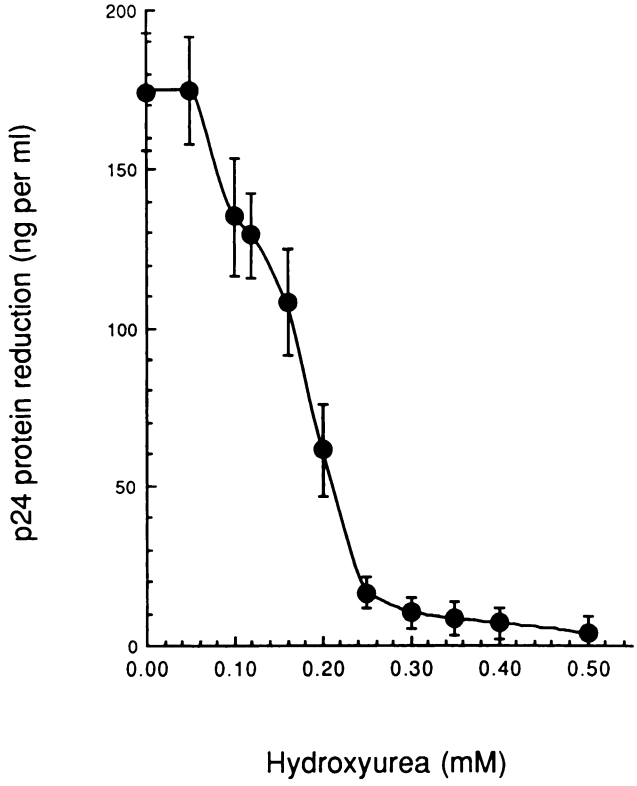

Fig. 1. Inhibition by HU of HIV-1 replication in PHA-stimulated PBM. PHA-stimulated PBM were exposed to HU at the indicated concentrations for 24 hr before infection. The HIV-1-containing medium was harvested at day 8 after infection and p24 protein production was determined by radioimmunoassay. Data represent mean values ± standard deviations of four independent experiments with four donors, with quadruplicate determinations in each experiment.

in PHA-stimulated PBM. The present study was designed to explore the effects of the combination of HU with dideoxynucleosides in this system and to determine the optimal scheduling of HU and ddI, the most effective of the HU/dideoxynucleoside combinations examined.

Experimental Procedures

Materials. All chemicals used were of reagent grade. HU and PHA were obtained from Sigma Chemical Co. (St. Louis, MO). ddI was supplied by Dr. Karl Flora, Developmental Therapeutics Program, National Cancer Institute. Recombinant interleukin-2 was purchased from R & D Systems (Minneapolis, MN). Radioimmunoassay kits for p24 Gag (group-specific antigen) protein were purchased from DuPont (Boston, MA). Sequenase enzyme (version 2.0) was obtained from United States Biochemical Corp. (Cleveland, OH). All oligodeoxynucleotides used as template-primers were purchased from Genosys Biotechnologies (Woodlands, TX).

Cells and virus. PBM were isolated from heparinized venous blood of healthy donors and were incubated for 72 hr with PHA (10 μg/ml) in RPMI 1640 medium supplemented with 15% heat-inactivated fetal calf serum, 15 units/ml recombinant interleukin-2, 4 mM L-glutamine, 50 units/ml penicillin, and 50 μg/ml streptomycin. An HIV-1 strain ($ERS104_{pre}$) was isolated, as described previously, from a patient with advanced HIV-1 infection, before antiviral therapy, at the National Cancer Institute (18).

Determination of anti-HIV-1 activity. PHA-stimulated PBM were plated in 24-well tissue culture plates at a density of 1×10^6 cells/well. Drugs were added in 2 ml of supplemented RPMI 1640 medium. After incubation for 24 hr cells were exposed to 2500 HIV-1 50% tissue

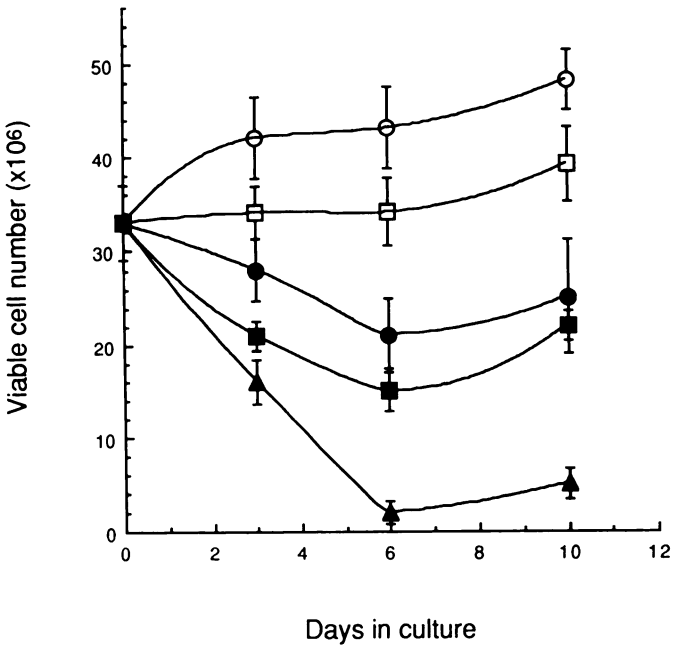

Fig. 2. Effect of HU on human PBM. PHA-stimulated PBM (33×10^6) were incubated with HU at different concentrations in 20 ml of culture medium. The cells were harvested at the indicated times and the viable (trypan blue-excluding) cells were counted. ○, No-HU control; □, 0.1 mM HU; ●, 0.5 mM HU; ■, 5 mM HU; ▲, 50 mM HU. The data represent the numbers of viable cells (average ± standard deviation) from three normal donors.

culture infective doses/well, and half of the culture medium was replaced with fresh culture medium containing the same concentrations of drugs on day 4 after infection. On day 8, the medium was harvested and the amount of p24 protein was determined by radioimmunoassay.

Analysis of intracellular dNTP pools in cells exposed to HU and ddI. Intracellular dNTP pools were quantified as described previously (19). A DNA polymerase assay for dNTPs described by Sherman and Fyfe (20) was adapted and, where necessary, combined with a dNTP reduction assay for correction for the interference from any ddNTPs present in the cell extract (21). Briefly, one dNTP present in excess was radiolabeled and the amount of radioactivity incorporated into DNA was proportional to the dNTP to be quantified. Sequenase enzyme (version 2.0) was used for this procedure. The Sequenase reaction mixture contained 50 mM Tris·HCl, pH 7.5, 10 mM $MgCl_2$, 5 mM dithiothreitol, 0.25 μM template-primer (the sequences of the oligodeoxynucleotides are described in Ref. 20), and 2.5 μM [^{3}H]dATP (15 Ci/mmol; for dCTP, dTTP, and dGTP determinations) or 2.5 μM [^{3}H]dTTP (15 Ci/mmol; for dATP determination). Cell extract (approximately 1×10^5 cells in 5 μl) was added to the reaction mixture to bring the total volume up to 50 μl. The polymerase reaction was carried out at 26° for 20 min, followed by spotting of 40 μl of the reaction mixture onto Whatman DE81 paper. The filters were extensively washed with 5% Na_2HPO_4, rinsed with distilled water and then 95% ethanol, and dried. The radioactivity on the filters was counted in a liquid scintillation counter.

Results

Effect of HU against HIV-1 replication. The inhibitory effect of HU alone on HIV-1 replication was evaluated in PHA-stimulated PBM using p24 Gag protein production as an endpoint. PHA-stimulated PBM were exposed to different concentrations of HU 24 hr before HIV-1 exposure. The culture medium was harvested on day 8 after infection and the production of HIV-1 p24 protein was examined by radioimmunoassay.

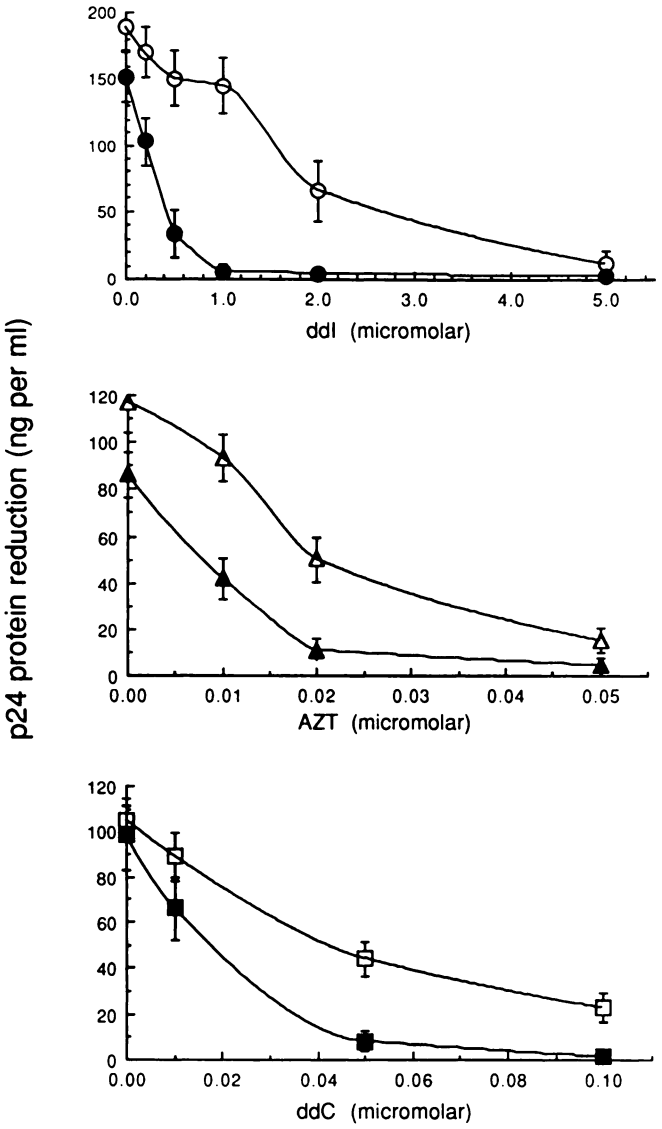

Fig. 3. Enhancement of anti-HIV-1 activities of ddI, AZT, and ddC by HU. PHA-stimulated PBM were incubated with ddI (*circles*), AZT (*triangles*), or ddC (*squares*), in the absence (*open symbols*) or presence (*closed symbols*) of 0.1 mM HU, for 24 hr before HIV-1 infection. The virus-containing medium was harvested at day 8 and the reduction of HIV-1 p24 protein was assayed by radioimmunoassay. Data represent mean values ± standard deviations for four donors (for the ddI study) or two donors (for the AZT and ddC studies), with quadruplicate determinations in each experiment.

As shown in Fig. 1, HU inhibited HIV-1 replication in a concentration-dependent manner. The IC_{90} of HU was 0.4 mM.

Because HU is toxic specifically to activated cells, we chose PHA-stimulated PBM for the cytotoxicity studies as well as for the HIV-1 inhibition studies. PBM were stimulated with PHA for 72 hr and exposed to various concentrations of HU. The viable cells were counted at different time points using the trypan blue exclusion method. As shown in Fig. 2, the cytotoxicity of HU was a function of the concentration of HU in the culture medium. After 10 days of exposure to 5 mM HU, there was approximately 54% inhibition of cell growth (from 48 × 10^6 to 22 × 10^6 cells). A cytostatic effect was observed at 0.5 mM HU (trypan blue-stained cells, <3%) (Fig. 2), a concentration that inhibited HIV-1 replication by >90% (Fig. 1).

TABLE 1

Potentiation by HU of ddI-, AZT-, and ddC-induced inhibition of HIV-1 p24 antigen production in PHA-stimulated PBM

PHA-stimulated PBM (1 × 10^6/assay) were infected with HIV-1 in the presence and absence of ddI (0.5, 1, 2, or 5 μM), AZT (10, 20, 40, or 80 nM), or ddC (20, 40, 80, or 160 nM), with different concentrations of HU as indicated. On day 8 after infection, the medium was harvested and HIV-1 p24 protein production was examined by radioimmunoassay. The no-drug control values were 179 and 150 ng/ml for donor A and B, respectively. HU at these concentrations did not yield inhibition of HIV-1 p24 protein production (data not shown); however, it reduced the IC_{90} values of ddI, AZT, and ddC. Values in parentheses represent the percentage of IC_{90} of the dideoxynucleosides, compared with the no-HU control. Data represent the means from two experiments, with triplicate determinations in each experiment.

	IC_{90} in culture		
	ddI	AZT	ddC
	μM	nM	nM
Donor A			
No HU	4.4 (100%)	101 (100%)	125 (100%)
With 0.01 mM HU	3.2 (73%)	75 (74%)	137 (109%)
With 0.04 mM HU	1.8 (41%)	46 (46%)	71 (57%)
With 0.08 mM HU	0.9 (20%)	35 (35%)	38 (30%)
Donor B			
No HU	1.0 (100%)	77 (100%)	68 (100%)
With 0.01 mM HU	0.9 (90%)	53 (69%)	74 (108%)
With 0.04 mM HU	0.7 (70%)	38 (49%)	62 (91%)
With 0.08 mM HU	0.2 (20%)	26 (34%)	40 (59%)

Potentiation of ddI activity against HIV-1. The hypothesis that HU, because of its selective effect against dATP pools, may potentiate the activity of ddI in activated PBM was examined by comparison of the anti-HIV-1 activity of ddI in the presence and absence of 0.1 mM HU. As shown in Fig. 3, 0.1 mM HU by itself showed only 30% or less inhibition of HIV-1; however, it yielded 45, 82, 97, 98, and 99% inhibition of HIV-1 replication when combined with ddI at 0.2, 0.5, 1.0, 2.0, and 5 μM, respectively. As a result, the IC_{90} value of ddI was reduced 6-fold (from 4.8 to 0.8 μM) (Fig. 3). This enhancement of ddI activity was observed at HU concentrations as low as 0.01 mM (Table 1). Furthermore, we found that 1 μM ddI, with only 23% antiviral effect, was able to inhibit 59%, 86%, and 96% of HIV-1 replication when combined with 0.02, 0.05, and 0.1 mM HU, respectively (Table 2). These effects of ddI brought about 5-fold reduction of the IC_{90} value of HU (from 0.4 to 0.08 mM) (Table 2). No cytotoxicity was observed with the HU and ddI combinations used in these experiments (Table 3).

Potentiation of AZT and ddC activities. The effects of HU on anti-HIV-1 activities of AZT and ddC were examined under conditions identical to those of the ddI experiments. The inhibition curves for AZT and ddC were shifted downward by the addition of 0.1 mM HU, although the potentiation was less than that for ddI (Fig. 3). These observations were in agreement with the IC_{90} studies, in which HU also enhanced the activities of AZT and ddC (Table 1) in the absence of cytotoxicity (Table 3).

Schedule dependence of HU action with ddI. Because HU action *in vivo* is relatively brief, we studied the antiviral activity of the combination of HU and ddI, with several drug schedules. The schedules, which are depicted schematically in Fig. 4, were designed to vary only in the time period of HU exposure, whereas 1 μM ddI was continuously present in the experiments. Table 4 shows that the presence of 1 μM ddI alone caused only 10–13% inhibition of viral p24 protein production, whereas the effects of 0.1 mM HU varied with different donors, with two of them showing no inhibition and one showing 41%

TABLE 2

Effect of HU concentration on ddI activity

PHA-stimulated PBM (1 × 10^6/assay) were infected with HIV-1 in the presence and absence of 1 μM ddI and different concentrations of HU as indicated. Values in parentheses represent the percentage of HIV-1 p24 protein produced, compared with the no-drug control. Data represent the means ± standard deviations for two donors, with triplicate determinations in each experiment.

	p24 protein production			
	0 mM HU	0.02 mM HU	0.05 mM HU	0.1 mM HU
	ng/ml			
No ddI	100.3 ± 10.1 (100%)	103.9 ± 6.2 (103%)	105.0 ± 11.6 (105%)	69.9 ± 4.9 (70%)
With 1 μM ddI	76.8 ± 7.7 (77%)	41.3 ± 17.3 (41%)	14.2 ± 7.4 (14%)	3.8 ± 2.1 (4%)

TABLE 3

Effects of the combination of HU with AZT, ddI, and ddC on the growth of PHA-stimulated PBM

PHA-stimulated PBM (1 × 10^6) were seeded in the presence or absence of 1 μM AZT, ddI, or ddC combined with different concentrations of HU as indicated. The viable cells were quantitated using the trypan blue exclusion method. Data represent the mean ± standard deviation from two experiments (two donors), with triplicate determinations in each experiment.

	Viable cell number (×10^6)									
	No drug	AZT + HU at			ddI + HU at			ddC + HU at		
		0.02 mM	0.05 mM	0.1 mM	0.02 mM	0.05 mM	0.1 mM	0.02 mM	0.05 mM	0.1 mM
Day 0	1.0 ± 0.1									
Day 4	2.6 ± 0.2	2.6 ± 0.3	2.5 ± 0.3	2.4 ± 0.2	2.8 ± 0.4	2.7 ± 0.3	2.4 ± 0.3	2.5 ± 0.4	2.4 ± 0.3	2.4 ± 0.4
Day 8	3.1 ± 0.3	3.1 ± 0.5	3.0 ± 0.2	2.8 ± 0.4	3.0 ± 0.3	2.9 ± 0.4	2.7 ± 0.4	2.9 ± 0.2	2.8 ± 0.4	2.5 ± 0.4

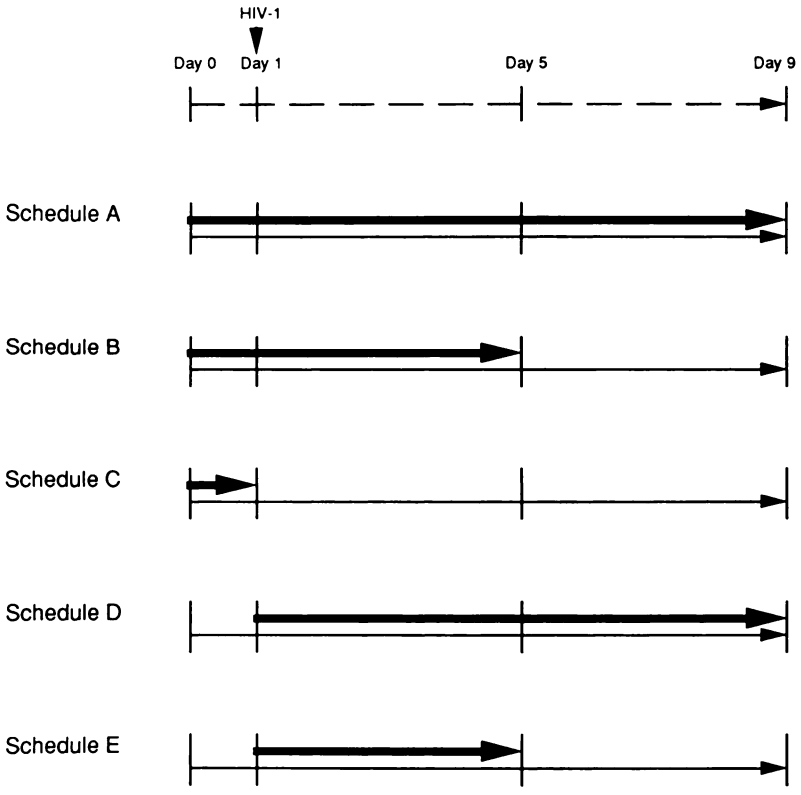

Fig. 4. Combinations of HU and ddI, with different schedules. The effects of the combination of HU (0.1 mM) and ddI (1 μM) were studied using different schedules. PHA-stimulated PBM (1 × 10^6/assay) were plated at day 0 and infected with 2500 HIV-1 50% tissue culture infective doses/10^6 cells at day 1. At day 5, the drug-containing medium was reconstituted. The reduction of HIV-1 p24 protein was examined at day 9. *Thick lines*, time periods of HU exposure; *thin lines*, exposure to ddI, which was uniformly present throughout the experiment. In schedule C, HU exposure was stopped at day 1 before HIV-1 infection by removal of HU-containing medium and addition of fresh medium containing only ddI. In schedules D and E, HU was added immediately before HIV-1 infection.

inhibition. As shown in Table 4, on the basis of HU exposure time and antiviral effect, schedule E (HU present from day 1, the viral adsorption, to day 4 after infection) produced the most profound inhibition (>81% inhibition compared with the no-drug control and 79% inhibition compared with the no-HU control). Interestingly, even the short HU exposure in schedule C (preincubation of cells with HU for 24 hr) inhibited p24 protein production by 56–79%.

In further investigation of the mechanism of the joint action of HU and ddI, we examined the effects of the combination on intracellular dNTP levels in PHA-stimulated PBM. Table 5 shows that, after 24-hr exposure to HU and ddI, the dATP pool was most profoundly decreased, from 1.76 to 0.29 pmol/10^6 cells, with minor reduction of other dNTP pools. The selective action on dATP was similar to that previously observed with HU alone (15) and that previously reported for HU in other mammalian cell culture systems (16, 17).

Discussion

Although it has been known for years that HIV-1 replication is dependent on the activation of host cells, this viral depend-

TABLE 4

Time and schedule dependence of joint action of HU and ddI against HIV-1 replication

PHA-stimulated PBM (1×10^6) were infected with HIV-1 in the presence or absence of 0.1 mM HU and 1 μM ddI, following the schedules described in Fig. 4. Values in parentheses represent the percentage of HIV-1 p24 protein produced, compared with the no-drug control.

	p24 protein production									
	No drug	HU	ddI	HU + ddI						
				Schedule A		Schedule B		Schedule C	Schedule D	Schedule E
	ng/ml									
Donor A	151.1 ± 13.6 (100%)	156.4 ± 11.4 (103%)	135.4 ± 6.1 (90%)	15.1 ± 3.4 (10%)		30.0 ± 8.9 (20%)		65.3 ± 9.2 (43%)	9.9 ± 4.6 (7%)	24.8 ± 9.8 (16%)
Donor B	165.2 ± 16.9 (100%)	171.6 ± 12.3 (104%)	142.9 ± 10.3 (87%)	19.0 ± 4.9 (12%)		43.2 ± 15.0 (26%)		64.4 ± 17.7 (39%)	15.3 ± 7.0 (9%)	30.0 ± 10.4 (18%)
Donor C	160.3 ± 10.5 (100%)	94.1 ± 6.0 (59%)	142.6 ± 9.0 (89%)	5.3 ± 1.5 (3%)		4.4 ± 1.3 (3%)		33.6 ± 12.9 (21%)	4.5 ± 1.1 (3%)	4.8 ± 3.0 (3%)

ency has only recently been taken as a target for the development of strategies against HIV-1 replication. In the present paper, we report that HU has a combined anti-HIV-1 activity with ddI, decreasing the IC_{90} value of ddI by 6-fold. In addition, HU was found to potentiate, although to a lesser extent, the activity of AZT and ddC. The HU concentrations active against HIV-1 replication were in the clinically achievable range (8). The currently recommended doses of HU for the long term treatment of chronic myelogenous leukemia are 40–80 mg/kg/day, which give peak plasma concentrations of 0.5–2.0 mM (11).

The inhibitory effect of HU against HIV-1 replication through reduction of intracellular dNTP pools is of particular interest. All of the anti-HIV-1 agents developed to date target viral proteins. However, a high rate of mutation is an intrinsic property of HIV-1 replication, and the virus is known to rapidly develop drug resistance (22). In contrast, as is typically the case with host proteins, ribonucleotide reductase does not mutate readily under physiological conditions. Our results show that the levels of host cell dNTPs can be controlled pharmacologically. The controlled dNTP pools have a selective inhibitory effect against HIV-1 replication with HU and ddI combinations. (i) Only cytostatic effects were observed at drug concentrations that inhibited viral replication by 90% (Table 3). (ii) HIV-1 replication was significantly more vulnerable to the brief exposure to HU and ddI than were the host cells (Table 4). (iii) HU has a short term action and is readily reversible with respect to toxicity. It is possible that transient interruption of ribonucleotide reductase function, with no consequent inhibition of host cell growth, effectively inhibited HIV-1 replication. This may provide a basis for scheduled intermittent regimens using HU and ddI in the therapy of AIDS and AIDS-related complex.

Several lines of evidence support the proposed mechanism of the HU/ddI combination specifically targeting HIV-1 proviral DNA synthesis. (i) The greatest inhibitory activity was exerted when HU was present after the viral adsorption period, apparently correlating with the time of proviral DNA synthesis (Table 4). (ii) Preincubation alone was able to inhibit >50% of subsequent HIV-1 replication (Table 4), whereas HU is not an inhibitor of HIV-1 reverse transcriptase (15), indicating the involvement of an indirect mechanism. (iii) The inhibition of p24 protein production was associated with the inhibition of the formation of intracellular dNTP pools, especially the dATP pool (Table 5). These results support the hypothesis that the target sites are the host ribonucleotide reductase (targeted by HU) and HIV-1 reverse transcriptase (targeted by ddATP generated from ddI).

The effects of HU in potentiating the action of pyrimidine dideoxynucleosides (AZT and ddC) are more complex, in view of the lesser effects of HU on pyrimidine dNTP pools than on dATP pools. Under some conditions, in fact, HU increases dTTP pools (16, 17), a change that would, in the absence of other factors, decrease rather than increase the activity of AZT. Karlsson *et al.* (23), who initially observed the stimulation by HU of AZT phosphorylation (and also 3′-fluorodeoxythymidine phosphorylation), thus proposed an alternate mechanism for the stimulation of phosphorylation of these compounds. Those authors suggested that the intracellular importation and subsequent phosphorylation of AZT or 3′-fluorodeoxythymidine are increased by a secondary effect of HU on ribonucleotide reductase, i.e., a HU-induced shift in balance in favor of (anabolic) pyrimidine nucleoside kinases (including thymidine ki-

TABLE 5

Effects of the combination of HU and dideoxynucleoside analogs on cellular dNTP pool sizes

PHA-stimulated PBM were exposed to 0.1 mM HU and/or 1 μM ddI, AZT, or ddC for 24 hr. Cells were extracted and dNTP levels were determined. Values in parentheses represent the percentage of dNTP pool sizes of cells exposed to drugs, compared with the pool size of no-drug controls. Data represent means ± standard deviations of two experiments, with triplicate determinations in each experiment.

	Pool size			
	dATP	dGTP	dCTP	dTTP
	pmol/10⁶ cells			
No drug	1.76 ± 1.09 (100%)	1.49 ± 4.30 (100%)	1.99 ± 0.40 (100%)	4.79 ± 0.37 (100%)
HU	0.86 ± 0.30 (49%)	1.59 ± 0.32 (106%)	2.12 ± 0.44 (106%)	4.37 ± 0.99 (91%)
ddI	1.30 ± 0.64 (74%)	1.29 ± 0.12 (87%)	1.52 ± 0.26 (76%)	4.00 ± 0.34 (84%)
AZT	1.74 ± 0.10 (99%)	1.60 ± 0.10 (107%)	1.95 ± 0.39 (98%)	4.94 ± 0.59 (103%)
ddC	1.67 ± 0.07 (95%)	1.64 ± 0.16 (110%)	0.79 ± 0.20 (40%)	5.94 ± 0.71 (124%)
HU + ddI	0.29 ± 0.03 (16%)	0.96 ± 0.23 (64%)	1.77 ± 0.18 (89%)	3.07 ± 0.37 (64%)
HU + AZT	0.92 ± 0.02 (52%)	1.42 ± 0.12 (95%)	1.96 ± 0.51 (98%)	4.94 ± 0.59 (103%)
HU + ddC	0.87 ± 0.04 (49%)	1.55 ± 0.14 (104%)	0.82 ± 0.19 (41%)	4.64 ± 0.37 (97%)

nase) over (catabolic) nucleotidases, so that on balance AZT or 3′-fluorodeoxythymidine phosphorylation is favored. This effect is apparently independent of an allosteric effect of dTTP on thymidine kinase, because the concomitant increase of dTTP levels by HU would tend to decrease rather than increase thymidine kinase activity. In the case of ddC phosphorylation, the stimulatory effect of HU noted by Balzarini *et al.* (24) is more readily explained, because dTTP allosterically activates deoxycytidine kinase, the enzyme responsible for the phosphorylation of ddC (23, 24).

In conclusion, we and others have observed that, of the cellular dNTP pools, the dATP pool is the most rapidly depleted by HU treatment. Because dATP and ddATP compete directly for reverse transcriptase and for incorporation into proviral DNA, it follows that ddATP-generating anti-HIV agents (e.g., ddI and 2′,3′-dideoxyadenosine) would show the greatest potential among dideoxynucleoside anti-HIV agents for therapeutic use in combination with HU.

Acknowledgments

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