A Pilot Study of Hydroxyurea among Patients with Advanced Human Immunodeficiency Virus (HIV) Disease Receiving Chronic Didanosine Therapy: Canadian HIV Trials Network Protocol 080

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To assess the in vivo short-term antiretroviral effect of hydroxyurea in human immunodeficiency virus (HIV)-infected persons chronically treated with didanosine (ddi), 26 patients with CD4 cell counts between 100 and 350 were enrolled in a 12-week, open-label pilot study and randomly assigned to receive 500 or 1000 mg/day hydroxyurea. Clinical status, laboratory toxicities, CD4 lymphocyte count, and HIV RNA plasma virus load were assessed weekly. Median declines from baseline of 0.02 and 0.63 log_{10} HIV-1 RNA copies/mL of plasma were observed for the 500- and 1000-mg/day groups, respectively ($P = .02$). CD4 cell counts did not change significantly with the addition of hydroxyurea; however, a small but statistically significant decrease in counts was observed during the washout phase. Both doses of hydroxyurea were well-tolerated. These results demonstrate a substantial decrease in plasma virus load when 1000 mg of hydroxyurea is administered over 1 month as adjunctive therapy to ddi among HIV-infected persons with 100–350 CD4 cells/mm$^3$.

To date, nucleoside analogues remain the cornerstone of treatment against human immunodeficiency virus (HIV) infection. Prolongation of the disease-free interval and survival have been clearly documented using zidovudine [1, 2] and didanosine (ddI) monotherapy [3, 4] and more recently when combinations of ddi and zidovudine or zidovudine and zalcitabine [5, 6] were used. Unfortunately, the benefits associated with currently available antiretroviral therapies appear to be time-limited, so the search for newer therapeutic strategies must continue.

In vitro studies have suggested that hydroxyurea can inhibit HIV-1 replication [7, 8]. This effect was substantially enhanced when hydroxyurea was combined with nucleoside analogues, particularly ddi [9, 10]. The definitive mechanism responsible for the in vitro synergy between hydroxyurea and ddi remains unclear. Hydroxyurea is known to inhibit the cellular enzyme ribonucleotide reductase [11]. This decreases the intracellular pool of the dNTPs, preferentially dATP, which in turn can lead to the enhanced uptake of ddATP, the active metabolite of ddi [9]. An alternative hypothesis suggests that hydroxyurea can act as a chelating agent of metalloproteins essential for viral replication [10]. Regardless of its ultimate mechanism of action, hydroxyurea in combination with ddi substantially decreases viral replication in vitro on activated and quiescent peripheral blood mononuclear cells [8].

Hydroxyurea’s clinical safety profile is well-characterized, as it has been in use for a number of years for the treatment of a variety of neoplasms [11]. More recently, this compound was shown to be of clinical benefit and generally well-tolerated when dose regimens of 15–35 mg/kg of body weight/day were used over 6 months for the treatment of sickle cell anemia [12].

We undertook the present pilot clinical trial in an attempt to characterize the short-term in vivo antiretroviral effect of two doses of hydroxyurea in patients with advanced HIV disease who had been chronically treated with ddi therapy.

**Methods**

**Study design.** This was a 12-week, open-label pilot study to determine the short-term in vivo antiretroviral effect of two doses of hydroxyurea as an adjuvant to ddi monotherapy. The study protocol is registered within the Canadian HIV Trials Network as study CTN-080. Eligible persons received ddi monotherapy for the initial 4 weeks of the study (baseline phase). They were then randomly allocated to receive 500 or 1000 mg/day hydroxyurea on an open-label basis plus continued ddi therapy for the next 4 weeks (hydroxyurea phase). They then received ddi monotherapy for the final 4 weeks of the study (washout phase).
Eligibility criteria. Study participants were required to have had a minimum of 3 months of continuous ddI therapy before enrollment and to have received a stable dose of ddI for the previous 4 weeks. Other eligibility criteria included 100–350 CD4 cells/mm\(^3\), age between 18 and 75 years, >2.5 g/L white blood cells, >1.0 g/L granulocytes, >100 g/L platelets, creatinine <1.5 times the upper limit of normal, and transaminases and alkaline phosphatase <3 times the upper limit of normal. Patients with uncontrolled AIDS-defining illnesses, those who were actively receiving chemotherapeutic agents, or those with active substance abuse were not eligible for the trial.

Subjects were required to have completed at least 3 of the first 4 baseline visits before progressing to the hydroxyurea phase of the study. Concomitant therapy with any agents possibly active against HIV other than ddI was not allowed for the 12 weeks of the study. Concomitant therapy with suppressive or prophylactic regimens against opportunistic infections was allowed as per contemporary guidelines.

Study medications. Study participants were instructed to continue taking ddI orally at a dose of 200 mg twice daily on an empty stomach throughout the study period. During the hydroxyurea phase, study participants were instructed to add hydroxyurea to their regimen. Hydroxyurea (Hydrea; Bristol-Myers Squibb, Wallingford, CT) was prescribed as 500-mg capsules. Patients were instructed to take hydroxyurea at least 3 h apart from ddI dosing. Patients randomized to 500 mg/day took one 500-mg capsule daily and those assigned to 1000 mg/day took 500-mg capsules twice daily.

Follow-up. Study participants were seen weekly for 12 weeks. Clinical assessment at each visit included a brief medical history, an assessment of compliance including pill count, a physical examination, and the administration of a targeted questionnaire regarding possible adverse effects related to study medications, including stomatitis, anorexia, nausea, vomiting, diarrhea, peripheral neuropathy, constipation, rash, dysuria, and alopecia. Adverse reactions were categorized as grade 1–4 according to the World Health Organization classification, and their relationship to the study medications was characterized as unlikely, possible, probable, or definite.

Laboratory measurements performed on a weekly basis included CD4 and CD8 cell counts, white blood cell count, platelet count, and hemoglobin, serum transaminases, serum lactate dehydrogenase, total bilirubin, creatinine, alkaline phosphatase, amylase, and uric acid measurements. Plasma virus load was also measured weekly using the Amplicor HIV-1 Monitor Assay (Roche Molecular Systems, Mississauga, Canada). All plasma virus load determinations were performed in a blinded manner at the Clinical Retrovirology Laboratory, British Columbia Centre for Excellence in HIV/AIDS.

Statistical methods. The primary end point of the study was the change in plasma viremia (HIV RNA copies/mL) during the hydroxyurea phase averaged over both treatment arms. The secondary end points included the difference between treatment groups with regard to change in plasma viremia and CD4 lymphocyte count during the hydroxyurea phase, clinical and laboratory drug-related adverse effects, and changes in these parameters during the washout phase.

The average monthly log_{10} plasma virus loads were calculated by taking the average of log_{10} virus load measurements weekly at weeks 1–4, weeks 5–8, and weeks 9–12 for the baseline, hydroxyurea, and washout phases, respectively. The median within-patient changes in plasma viremia were compared between the baseline and hydroxyurea phases for the entire study population with a Wilcoxon signed rank test. The difference in the change in median log_{10} virus load between the baseline and hydroxyurea phases between treatment groups was tested with a Wilcoxon rank sum test.

The relationships between the change in mean plasma virus load from the baseline to the hydroxyurea phase and the duration of prior ddI therapy, the duration of prior zidovudine therapy, the CD4 cell count at baseline, and the level of plasma viremia at enrollment were assessed using a Spearman’s correlation coefficient. Generalized estimating equation regression models, which allow for correlated observations within individuals [13], were used to estimate the effects of each dose of hydroxyurea on plasma virus load and CD4 cell counts.

Sample size estimation. On the basis of the known short-term variability of plasma virus load, we identified a change in plasma virus load of ≥0.5 log_{10} as indicative of a definite treatment effect [14]. Patients were said to have “responded” to therapy if the mean log_{10} virus load during the hydroxyurea phase was at least 0.5 log_{10} copies lower than the mean virus load during the baseline phase.

A sample size of 12 patients per arm was calculated as sufficient to detect a change of 0.5 log_{10} copies/mL in the mean log virus load between the baseline and hydroxyurea phases of the study, assuming the SD of the change in log virus load to be 0.5 log_{10} copies/mL, a significance level of .05, and a power of 80%.

Results

Thirty-one patients were enrolled between 15 March and 15 June 1995. Five participants were deemed to be ineligible for randomization during the baseline phase of the study because of CD4 cell counts outside the range (n = 2), lack of compliance (n = 1), newly developed gastrointestinal intolerance to ddI (n = 1), and worsening hepatic function (n = 1). A total of 26 patients were therefore randomized and are reported here as the study group.

All but 2 of 26 patients were men, the mean age was 44 years, the mean baseline CD4 cell count was 239 cells/mm\(^3\), the median baseline plasma virus load was 4.1 log_{10} copies/mL, and the median duration of prior ddI therapy at enrollment was 16 months (range, 3–48). All study participants had been previously treated with zidovudine: 20 of them were treated until enrollment, while the other 6 had stopped zidovudine therapy between 17 and 61 months before enrollment. As shown in table 1, baseline characteristics were not statistically significantly different between treatment groups. Twenty-two patients completed all 12 visits; 2 missed 1 visit, 1 missed 2 visits, and 1 missed 3 visits, all in the washout phase. One subject in the 500-mg/day group discontinued ddI at week 9 because of newly developed gastrointestinal intolerance but completed the remaining visits while receiving zidovudine monotherapy.
Table 1. Baseline patient characteristics in a study of hydroxyurea and ddI treatment.

| Hydroxyurea dose | 500 mg (n = 13) | 1000 mg (n = 13) | P  
|------------------|-----------------|-----------------|-----
| Male, no. (%)    | 13 (100)        | 11 (84.6)       | .48 |
| Age, years, mean | 42 (31–57)      | 44 (33–61)      | .47 |
| CD4 cell count/mm³, median (range) | 228 (103–400) | 230 (103–335) | .61 |
| Log₁₀ plasma virus load, copies/mL of plasma, median (range) | 3.7 (1.5–5.3) | 4.1 (1.9–5.3) | .72 |
| Karnofsky score, median (range) | 90 (0) | 90 (0) | .99 |
| Months of prior ddI, median (range) | 13 (3–43) | 17 (5–48) | .84 |
| Months of prior zidovudine, median (range) | 25 (1–71) | 21 (5–36) | .74 |
| Risk group, no. (%)  
| Homosexual/bisexual | 12 (92.3) | 11 (84.6) | .99 |
| Heterosexual contact | 1 (7.7) | 1 (7.7) | .99 |
| Unknown          | 0               | 1               | .99 |

* Represents average of weeks 1–4.

Figure 1 shows the weekly median change in virus load from baseline by treatment group. The median decline in log₁₀ plasma viremia from the mean level during the baseline phase to the mean level during the hydroxyurea phase for the entire study group was 0.21 log₁₀ copies/mL (P = .06). The median declines in log₁₀ plasma virus load for the 500- and 1000-mg/day groups were 0.02 and 0.63 log₁₀ copies, respectively. These were statistically significantly different (P = .03).

Eight of the 26 subjects had declines > 0.5 log₁₀ in mean plasma virus load while receiving hydroxyurea: 1 and 7 in the 500- and 1000-mg/day groups, respectively (P = .03). For 16 of the 26 subjects, the maximum difference between the mean plasma viremia at baseline and any single visit while receiving hydroxyurea was > 0.5 log₁₀ (7 in the 500-mg/day group, 9 in the 1000-mg/day group, P = .69). For 9 of the 26 subjects, the maximum difference between the mean log₁₀ plasma virus load during baseline and any single visit while receiving hydroxyurea was > 1.0 log₁₀: 2 and 7 in the 500- and 1000-mg/day groups, respectively (P = .10).

Maximum changes from baseline in plasma virus load were seen at week 5 (n = 1), 6 (n = 3), 7 (n = 11), and 8 (n = 8). Three patients (all in the low-dose arm) showed no decrease in plasma virus load while receiving hydroxyurea. Maximum individual mean changes from baseline to weeks 5–8 were −0.6 log₁₀ in the 500-mg/day group and −2.4 log₁₀ in the 1000-mg/day group.

The change in mean plasma viremia levels from baseline was unrelated to the prior duration of ddI therapy (Spearman’s correlation coefficient = .06, P = .78), prior duration of zidovudine therapy (Spearman’s correlation coefficient = .06, P = .78), mean baseline CD4 cell count (Spearman’s correlation coefficient = .19, P = .34), and mean baseline plasma viremia (Spearman’s correlation coefficient = −.11, P = .61). However, there was a statistically significant correlation between duration of prior zidovudine therapy and the maximum decrease in plasma viremia while receiving hydroxyurea (Spearman’s correlation coefficient = .46, P = .03).

We further explored the effect of hydroxyurea on virus load using a generalized estimating equation model [13]. A fixed correlation matrix was specified, which assumed that the correlation between any two pairs of viremia measurements was 0.80 − 0.01 × number of weeks between the pairs of measurements, as suggested by the data. A statistically significant difference was shown between treatment groups (500 vs. 1000 mg/day hydroxyurea) with respect to the change in plasma viremia from baseline while receiving study drug (estimated difference = 0.6 log₁₀ copies/mL, P = .01). This model confirms a greater virus load decrease when 1000 mg/day of hydroxyurea was used.

As shown in table 1, the median CD4 cell count at baseline was 228 and 230 for the 500- and 1000-mg/day groups (P = .61), respectively. As shown in figure 1, CD4 cell counts remained stable during the hydroxyurea phase (P = .45). A small but statistically significant decrease in CD4 cell counts was noted between washout and baseline—a total of 24 cells/mm² for all patients (P = .004). No statistical difference was found between dose groups (P = .66).

A total of 6 patients, 5 in the 500-mg/day and 1 in the 1000-mg/day groups, developed oral candidiasis during the study period. One patient (in the 1000-mg/day group) developed Pneumocystis carinii pneumonia and another patient (in the 500-mg/day group) developed esophageal candidiasis during the washout phase. There were no statistically significant changes in weight or Karnofsky score during the study. No deaths occurred during the study period.

All adverse events of any severity at least possibly related to hydroxyurea are listed in table 2. All adverse effects were mild to moderate and did not preclude continued hydroxyurea dosing. No statistically significant association was found between any or all adverse events and dose of hydroxyurea.

**Discussion**

Our data demonstrate a short-term statistically significant decrease in plasma viremia when 1000 mg of hydroxyurea was added to continued ddI therapy in HIV-infected persons with 100–350 CD4 cells/mm³. We also demonstrated a dose-related effect of hydroxyurea on plasma viremia, with a median decline of 0.63 log₁₀ copies of HIV RNA/mL among patients receiving 1000 mg/day and 0.02 log₁₀ copies of HIV RNA/mL among patients receiving 500 mg/day. Furthermore, despite extensive prior antiretroviral therapy, including ddI, maximum mean plasma virus load decreases associated with the addition of hydroxyurea reached −2.4 log₁₀ copies of HIV RNA/mL with
doses of 1000 mg/day. Finally, the rebound in plasma virus load during the washout phase further supports the drug-related nature of the observed changes in plasma virus load while receiving hydroxyurea treatment.

Our data provide controlled evidence of an in vivo antiretroviral effect of hydroxyurea. Previous reports consisted of in vitro studies or limited uncontrolled patient series [15–18]. Based on the available evidence, we limited our study to the use of hydroxyurea in the context of ddI therapy. Further studies will be required to clarify whether the in vivo antiretroviral effect of hydroxyurea is also present within the context of other drug combinations.

Our patient population was predefined as to minimize known sources of short-term variability in plasma virus load. Hence, we selected patients who had advanced disease but were clinically stable. Further, we required a minimum of 3 months of ddI therapy prior to enrollment and we allowed no other treatments with potential effect on plasma virus load during the study to avoid the potential confounding effect of such co-interventions. Also, we monitored patients during the 1-month baseline period while receiving ddI to ensure the absence of any ongoing effects of this therapy on plasma virus load.

While our eligibility criteria minimized baseline variability in plasma virus load, they also generated a conservative bias regarding the antiretroviral effect of hydroxyurea. Prior exposure to ddI and zidovudine is likely to have minimized treatment response. In this context, if the ability of hydroxyurea to affect virus load is mediated through the enhancement of ddATP uptake, as previously suggested, extensive prior ddI therapy may have minimized therapeutic response in our setting [9]. It is noteworthy that HIV resistance to ddI, as demonstrated by a point mutation at codon 74 of the HIV reverse tran-
Hydroxyurea was generally well-tolerated in our study. No treatment-limiting adverse events were observed. Given the short-term design of our protocol, caution must be exercised when extrapolating this to possible long-term use of this agent. In a controlled clinical trial to assess the role of hydroxyurea in patients with sickle cell anemia, most of 152 subjects assigned to take an escalating dose of hydroxyurea starting at 15 mg/kg of body weight/day temporarily discontinued hydroxyurea because of bone marrow depression over a follow-up period of 21 months [12]. Limited bone marrow reserve in HIV-infected persons might lower the threshold for myelotoxicity to cytostatic agents [22].

Our results show that a short course of hydroxyurea can have an antiretroviral effect in patients chronically treated with ddI regardless of the length of prior therapy. Combinations of nucleoside analogues are currently recommended for the treatment of HIV infection [5, 6, 23]. In this context, the potential adjunctive effect of hydroxyurea merits further evaluation.

In summary, our results demonstrate a substantial decrease in plasma virus load when hydroxyurea is administered as adjunctive therapy to ddI among HIV-infected persons with 100–350 CD4 cells/mm³. We demonstrated a median decline in plasma viremia of 0.63 log₁₀ HIV RNA copies/mL when a dose of 1000 mg/day hydroxyurea was used. Maximum mean virus load decline reached −2.4 log₁₀ HIV RNA copies/mL with this same dose. CD4 cell counts, however, did not change significantly while patients were receiving hydroxyurea. Hydroxyurea was well-tolerated in this patient group. Our findings suggest that the potential clinical role of hydroxyurea as adjunctive therapy should be further evaluated.

Acknowledgments

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Table 2. Number of patients with adverse events of any severity at least possibly attributed to hydroxyurea.

<table>
<thead>
<tr>
<th>Hydroxyurea dose</th>
<th>500 mg (n = 13)</th>
<th>1000 mg (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nausea</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Headache</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Dysuria</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Stomatitis</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Other*</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>7</td>
</tr>
</tbody>
</table>

* Rashes, peripheral neuropathy, vomiting, anorexia, hyperamylasemia, thrush.

scriptase, has been shown to occur in 56% of patients after 6 months of ddI therapy [19]. Alternatively, zidovudine resistance may also have affected ddI response as shown in ACTG protocol 116B/117. Although we did not screen for point mutations at codon 74 or zidovudine resistance at baseline, it is likely that this was present in a number of subjects given the extensive prior exposure to zidovudine and ddI in our study population.

Plasma viremia decreases of >0.5 log₁₀ copies of HIV RNA/mL are generally accepted as indicative of a treatment effect [14]. Recently, Yerly et al. [20] reported that a decrease in plasma viremia between 0.63 and 0.82 log₁₀ HIV RNA copies/mL after 1 month of ddI monotherapy was predictive of improved survival, independent of an initial diagnosis of AIDS or baseline CD4 cell counts. Interestingly, our data demonstrate plasma viremia reductions of a similar magnitude among patients chronically exposed to ddI. We speculate that a greater antiretroviral effect could occur if ddI and hydroxyurea are administered together to ddI-naïve persons. We further speculate that hydroxyurea may help to prolong or restore the documented benefits associated with ddI therapy [3–5].

CD4 cell counts remained stable during the hydroxyurea phase of the study. Paradoxically, changes in CD4 cell counts failed to parallel reduction in plasma viremia. A similar dissociation between plasma virus load and CD4 cell response was recently observed by Clotet et al. [15] in an uncontrolled series in which patients received ddI and hydroxyurea for a 3-month period. This may relate to the effect of hydroxyurea on cell proliferation, which could have interfered with the rapid CD4 cell turnover observed in asymptomatic stages of HIV disease [21]. Whether this effect also contributes to the measured effect of hydroxyurea on plasma virus load in vivo remains to be further assessed. Given the dissociation between the virus load and CD4 cell count effect of hydroxyurea, it would be premature to conclude that the described virus load effect will lead to a clinical benefit. This issue will only be resolved on completion of ongoing long-term clinical trials.

References


