Rationale for the Use of Hydroxyurea as an Anti–Human Immunodeficiency Virus Drug

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Hydroxyurea has been extensively used in medical practice, mainly for treating chronic myelogenous leukemia, sickle cell anemia, and other diseases. In light of its ability to inhibit DNA synthesis and to induce cell cycle arrest through inhibition of ribonucleotide reductase, the effects of hydroxyurea on replication of human immunodeficiency virus type 1 (HIV-1) have been investigated. In vitro hydroxyurea has been shown to block HIV-1 reverse transcription and/or replication in quiescent peripheral blood mononuclear cells (PBMC) and macrophages. Hydroxyurea was also found to be synergistic with the nucleoside reverse transcriptase inhibitor didanosine and to inhibit HIV-1 replication in activated PBMC; this inhibition may be due to a reduction in deoxynucleoside triphosphate pool sizes. Finally, hydroxyurea has been shown to sensitize didanosine-resistant mutants. Hydroxyurea may therefore be useful for limiting the spread of didanosine-resistant HIV-1 variants. The favorable toxicity profile of hydroxyurea and the lack of significant overlapping toxicities with some of the nucleoside reverse transcriptase inhibitors, as well as their distinct mechanisms of action, have provided further rationale for use of these agents in combination therapies.

Therapy for HIV type 1 (HIV-1) infection is currently based on highly active antiretroviral therapies (HAARTs), which are combinations of drugs (reverse transcriptase [RT] inhibitors and protease inhibitors) that decrease plasma viremia to undetectable levels. Because these drugs tend to generate resistance, maximal virus suppression is required to avoid the emergence of drug-resistant mutants and to ensure long-term efficacy. Unfortunately, complicated dosing schedules, toxicity, and high cost make access to and compliance with many HAART regimens difficult for most patients. Viral escape occurs in many cases, leaving individuals with multidrug-resistant and/or cross-resistant variants of HIV. Transmission of these mutant viruses has also been documented. Salvage therapy for these patients is an extremely difficult task, because several antiretroviral drugs exhibit overlapping profiles of resistance. Therefore, development of new classes of drugs that are not prone to resistance and have different mechanisms of action is essential for long-term suppression of HIV.

Hydroxyurea is used most often for cytoreduction during the initial phase of chronic myelogenous leukemia but is also used for treating polycythemia vera and psoriasis. In addition, it has demonstrated modest activity against a variety of solid tumors [1–5]. Most recently, hydroxyurea has demonstrated activity in the treatment of sickle cell anemia by increasing the production of fetal hemoglobin, which reduces hemolysis in patients with this disease [6]. Hydroxyurea exerts its cytostatic effect through inhibition of ribonucleotide reductase—the rate-limiting enzyme responsible for the conversion of ribonucleotides to deoxyribonucleotides, which are essential for DNA synthesis. As a result, cellular division is arrested in the S phase [5]. This and other biological effects have stimulated interest in hydroxyurea for the treatment of several diverse diseases, including those mediated by DNA viruses [7–9]. Importantly, its excellent oral bioavailability [1] and limited scope of adverse effects (primarily modest myelosuppression) further strengthen its potential as a clinically useful therapy. The favorable resistance profile of hydroxyurea and the reduction of resistance to some nucleoside analogues make this drug a potential candidate for durable suppression of HIV.

HIV-1 Replication and the Cell Cycle

Soon after the discovery of HIV-1 as the etiologic agent of AIDS, it became clear that the cell cycle itself affects the replication of HIV-1 [10]. Quiescent CD4+ T lymphocytes and other nondividing cells, such as macrophages, are believed to constitute a major target for HIV-1 replication. However, the virus is unable to complete its replication cycle in nondividing CD4+ T lymphocytes; full production of the virus is observed only in dividing CD4+ T cells [11, 12]. The inability of resting cells to produce infectious virus is thought to be due to the low level of activity of the enzymes that generate the deoxynucleoside triphosphates (dNTPs) required for DNA synthesis. We evaluated this hypothesis by measuring the concentrations of dNTPs both in untreated or quiescent peripheral blood mononuclear cells (PBMC) and in PBMC stimulated with phytohemagglutinin [13]. We found that the concentration of...
dNTPs exceeded the Michaelis constant required for HIV-1 RT activity only in the stimulated cells. When PBMC were infected with HIV-1, similar results were obtained. We also tested the activity of HIV-1 RT by using the dNTP concentrations that were determined for quiescent and activated PBMC. As anticipated, the activity of HIV-1 RT under quiescent conditions was profoundly lower than that observed when concentrations of dNTPs corresponding to those found in activated cells were used.

**Effect of Hydroxyurea on HIV-1**

The relationship between the dNTP concentration and the level of HIV-1 RT activity, coupled with the impact that hydroxyurea has on the conversion of ribonucleotides to deoxyribonucleotides, prompted our group to investigate whether hydroxyurea could reduce dNTP concentrations to a level similar to those found in quiescent cells and thereby to reduce HIV-1 DNA synthesis.

**Depletion of essential nucleotides and inhibition of HIV-1 DNA synthesis.** In cells treated with hydroxyurea, deoxyadenosine triphosphate (dATP), deoxyguanosine triphosphate, deoxyxycytidine triphosphate (dCTP), and deoxymethidine triphosphate (dTTP) are depleted, thereby depriving cells of the essential triphosphorylated nucleotides for DNA synthesis [14]. Studies have revealed that dATP is more extensively depleted after exposure to hydroxyurea than the other nucleotides. Our initial in vitro investigations in which PCR analysis was used to measure HIV-1 DNA synthesis confirmed that hydroxyurea was able to block HIV-1 replication in quiescent and activated PBMC and in macrophages from normal individuals, as well as in activated PBMC from HIV-1–infected individuals [13, 15, 16]. Inhibition appears to be dose-dependent. Although both 0.1 and 1.0 mM hydroxyurea resulted in slower and less efficient DNA synthesis, the higher concentration produced almost complete inhibition. A striking feature of this inhibition was that it persisted after hydroxyurea was withdrawn [15]. In addition, no rebound effect was observed after incomplete inhibition with the lower concentration.

**Cytostatic effect on CD4+ T cells.** As discussed later in this article, HIV-1 is only able to replicate in dividing CD4+ T cells. Therefore, the cytostatic effect of hydroxyurea may also have a beneficial effect on the course of HIV infection as a result of reduction of cellular division of CD4+ T lymphocytes and subsequent reduction of viral replication.

**Nucleoside Analogues and HIV-1 Replication**

The available dideoxynucleoside RT inhibitors—didanosine, lamivudine, stavudine, zalcitabine, and zidovudine—inhibit the RT enzyme by competing with naturally occurring dNTPs for incorporation into the growing viral DNA chain. Each of these agents is inactive until it is phosphorylated by intracellular kinases and converted to the active triphosphate metabolite. Once incorporated, these nucleoside analogues act as chain terminators, prohibiting further chain elongation. Although they share a common framework of action, the nucleoside analogues do not all compete with the same dNTPs. Stavudine and zidovudine compete with dTTP, didanosine competes with dATP, and zalcitabine competes with dCTP. This difference is significant because the intracellular nucleotide pools play a critical role in the activity of these nucleoside analogues[17]. A decrease in the intracellular level of dATP, for example, would be expected to increase the ability of didanosine to inhibit RT but have little effect on the other RT inhibitors.

**Hydroxyurea Combined with Dideoxynucleoside RT Inhibitors: Complementary Actions and Potential Synergy**

The ability of hydroxyurea to deplete dNTP pools suggests that it might enhance the activity of nucleoside analogues by increasing their ability to compete with endogenous nucleotides for incorporation into the HIV-1 chain (table 1)[13, 17]. These results are described in the sections that follow. We also postulated that the combination of hydroxyurea with an RT inhibitor may reduce the emergence of resistant strains of the virus. In addition, the excellent distribution of hydroxyurea throughout the body [1] might enhance inhibition of HIV-1 in sequestered sites, such as the brain. In fact, hydroxyurea distributes rapidly to tissues [18] and readily enters the CSF [19].

The hypothesis that the combination of hydroxyurea with a nucleoside RT inhibitor might increase inhibition of HIV-1 replication was first tested in activated PBMC from healthy individuals [15]. Cells were infected in vitro with HIV-1 and were incubated in the presence of varying concentrations of hydroxyurea plus didanosine or zidovudine. The combination of didanosine plus hydroxyurea completely blocked production of HIV-1 p24 antigen (>99.9%). The inhibitory action was found to be attributable to specific antiretroviral effects rather than to cytostatic effects. We found the combination of didanosine plus hydroxyurea exhibited mathematically proven synergy by calculating the best fit of the data to the robust potentiation model using the COMBO program package [15]; didanosine plus hydroxyurea was several times more potent than hydroxyurea plus zidovudine.

**Table 1. Potential additive or synergistic effects of hydroxyurea on the activity of nucleoside reverse transcriptase inhibitors (NRTIs) against HIV type 1 (HIV-1).**

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<tr>
<th>Effect of Hydroxyurea</th>
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<td>Inhibition of DNA synthesis, slowing production of viral DNA</td>
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<tr>
<td>Enhanced NRTI penetration</td>
<td>Depletion of dNTP pools, thereby increasing the competitive ability of NRTIs for incorporation into the HIV-1 DNA chain</td>
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<td>Enhanced NRTI phosphorylation</td>
<td>Enhancement of NRTI phosphorylation, reducing resistance to NRTIs</td>
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<td>Reduced cellular division of CD4+ T lymphocytes</td>
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NOTE. dNTP, deoxynucleoside triphosphate.
Early in vitro studies reported by Gao et al. [16], in which hydroxyurea was combined with various dNTP analogues, also found that hydroxyurea potentiated anti–HIV-1 activity. They observed that the combination of a nucleoside RT inhibitor and hydroxyurea reduced the concentration of nucleoside analogues required to achieve 90% inhibition and that the potentiation was greater with didanosine than with either zalcitabine or zidovudine. The mechanism of this potentiation appears to be a reduction of dNTP concentrations. The most profound inhibition of the dATP pool was observed in cells treated with didanosine plus hydroxyurea [13, 16]. PBMC were stimulated with phytohemagglutinin and were treated with 0.1 mM hydroxyurea and 0.1 mM didanosine, zidovudine, or zalcitabine for 24 h before determination of dNTP pool sizes. In cells exposed to both didanosine and hydroxyurea, the dATP pool size was 16% of that measured in cells without exposure to either drug. The effect of the other nucleoside RT inhibitors was more modest because they are either cytosine analogues (competing with dCTP) or thymidine analogues (competing with dTTP).

The dATP pool sizes with zidovudine plus hydroxyurea and zalcitabine plus hydroxyurea were 52% and 49% of the control cells, respectively [16]. These data suggested that combination therapy with hydroxyurea plus didanosine might produce more profound anti–HIV-1 effects than combinations that employ other agents. This hypothesis was substantiated by in vitro work that showed that didanosine plus hydroxyurea was more potent than hydroxyurea plus zidovudine or hydroxyurea plus zalcitabine [3, 15, 16]. Additional in vitro studies confirmed that the synergistic inhibition of HIV-1 by didanosine plus hydroxyurea occurred at concentrations of didanosine that were readily achievable in vivo [3]. Statistical models have also given evidence for the synergistic effects of didanosine plus hydroxyurea [15].

Enhanced effect on nucleoside RT inhibitor phosphorylation. As mentioned previously, nucleoside RT inhibitors must be converted to their triphosphate form by intracellular kinases before being incorporated into the DNA chain. The arrest of the cell cycle in the S phase by hydroxyurea augments the activity of these kinases, and in vitro studies have shown greater intracellular uptake and phosphorylation of the nucleoside RT inhibitors in the presence of this agent [20, 21].

Modulation of the immune system. After initiation of antiretroviral therapy, there is typically a late increase in proliferating CD4+ T cells that results in an increase of CD4+ T cell counts in the periphery. These proliferating CD4+ T cells provide a favorable environment for HIV replication and might contribute to viral rebound during antiretroviral therapy. The cytostatic effect of hydroxyurea appears to blunt the increase of CD4+ T cells in the peripheral blood, which may influence the long-term antiviral activity of hydroxyurea plus didanosine–containing therapies, as well as reduce the likelihood of viral rebound in some patients after interruption of therapy [22–26]. In fact, a drug-induced decrease in the number of activated T lymphocytes (cells that can support a fully productive HIV infection cycle) might decrease overall production of the virus. More simply, “no cell division/activation equals no virus replication.” Several mathematical models predict this possibility [27–31]. A large clinical trial (AIDS Clinical Trials Group Study 343 [32]) recently supported the hypothesis that the greatest increase in the number of “prey” (CD4+ T lymphocytes) results in the highest risk for the return of “predators” (HIV virions).

In addition, down-modulation of CD8+ T cells by hydroxyurea may reduce excessive activation of these cells, which may be associated with immunopathologic effects. It has been shown that T lymphocyte turnover during HIV-1 infection is accompanied by shortening of the length of the telomeres in CD8+ T cells but not in CD4+ T cells, which indicates the potential for exhaustion of HIV-1–specific CD8+ T cells and, potentially, bystander CD8+ T cell populations [33–35]. A similar phenomenon occurs in the context of chronic noncytolytic infection with other viruses. Debate remains whether the decline in CD4+ T cells during HIV-1 infection is primarily caused by a cytolytic effect of the virus or by immunopathologic mechanisms, including lysis of infected cells by cytolytic CD8+ T lymphocytes. However, it is conceivable that excessive CD8+ T cell activation in response to continued viral replication results in both CD8+ T cell exhaustion and depletion of CD4+ T cells, which have been depleted by lysis of cytolytic CD8+ T lymphocytes. The use of an agent like hydroxyurea that both down-modulates CD8+ T cell activation and inhibits HIV-1 replication may prevent both exhaustion of CD8+ T cell populations and depletion of CD4+ T cells. CD4+ T cell depletion may be prevented regardless of whether depletion is due to a direct lytic effect of the virus (as a result of the effect of hydroxyurea on cellular and viral DNA replication) or to the lytic effect of cytolytic CD8+ T lymphocytes (as a result of the decreased activation of CD8+ T lymphocytes).

Reduction of HIV-1 Resistance to Nucleoside Analogues

We have recently demonstrated that HIV-1 variants resistant to didanosine are significantly more susceptible in the presence of hydroxyurea [22]. This finding was observed when low concentrations of hydroxyurea, together with concentrations of zidovudine or didanosine that are routinely achieved in vivo, were used. Lower levels of the nucleoside analogues were also needed to block the replication of nucleoside-resistant HIV-1 variants when hydroxyurea was present. These data suggest that hydroxyurea may be useful to limit the spread of didanosine-resistant variants of HIV-1 and to support the use of this drug in combination with nucleoside analogues, both after resistance has developed, and to limit the emergence of new mutants.

In fact, ribonucleotide reductase (the principal target of hydroxyurea) is not prone to mutations like the viral proteins. Ribonucleotide reductase resistance to hydroxyurea has never
been reported during 35 years of clinical experience. In addition, hydroxyurea can probably delay the onset of resistance to nucleoside analogues, simply by reducing the viral replication rate and therefore slowing the rate of mutation. In addition, lowering the levels of intracellular nucleoside triphosphates that the nucleoside analogues must compete against may be sufficient to make a genotypically resistant virus phenotypically susceptible.

One mechanism of nucleoside RT inhibitor resistance appears to be a reduction in their intracellular conversion to the active triphosphate form. Therefore, the augmentation of nucleoside RT inhibitor phosphorylation by hydroxyurea, which was described elsewhere [20, 21], may also reduce resistance to these agents.

Practical Considerations in the Use of Hydroxyurea in Combination Therapy for HIV Infection

Presently, hydroxyurea (Hydrea; Bristol-Myers Squibb, Nutley, NJ) is used at a dosage of 500 mg b.i.d., which is a significantly lower concentration than that used for treating leukemia. Recently, however, new doses of hydroxyurea have become available as 200-, 300-, and 400-mg capsules (Droxia; Bristol-Myers Squibb). To determine the optimal dose and daily schedule of hydroxyurea in combination therapies for HIV-1 infection, we have initiated a controlled clinical trial involving 225 patients (RIGHT 702).

The inherent toxicities associated with both hydroxyurea (primarily myelosuppression) and the dideoxynucleoside RT inhibitors (e.g., neuropathy associated with stavudine) raised concerns that this combination might be tolerable. However, the lack of significant toxicities that overlap those of some nucleoside RT inhibitors (i.e., didanosine) and the distinct mechanisms of action have led us to believe that these agents could be safely used in combination. It is important to note that there is overlapping bone marrow toxicity between hydroxyurea and zidovudine.

Conclusions

In vitro investigations have demonstrated that hydroxyurea can reduce the synthesis of viral DNA. Its inhibition of ribonuclease reductase and depletion of deoxyribonucleotide pools suggested that it would have additive or synergistic effects when used in combination with nucleoside RT inhibitors such as didanosine. Indeed, preclinical studies have confirmed that hydroxyurea reduces the concentration of nucleoside RT inhibitor necessary to inhibit viral DNA synthesis. Because HIV-1 replicates only within actively proliferating cells and not within resting cells, the cytostatic effect of hydroxyurea on CD4+ T lymphocytes, the host cells for HIV-1, may produce additional beneficial effects. Finally, the favorable toxicity profile of hydroxyurea and the lack of significant overlapping adverse effects with some nucleoside RT inhibitors make it an excellent candidate for combination therapy. The clinical significance of these intriguing biological effects is now being investigated in clinical trials (see the article by Zala et al. [36] in this supplement).

References


