Target cell availability and the successful suppression of HIV by hydroxyurea and didanosine

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Introduction

Surprisingly, immunosuppressive treatment can enhance the efficacy of conventional HIV-1 antiretroviral treatment, and can be beneficial for HIV-1-infected patients. This argues for a role of target cell availability in limiting the HIV-1 infection, and is in agreement with mathematical models suggesting that immunosuppression may limit the outgrowth of drug-resistant escape mutants. Immunosuppressive drugs like hydroxyurea (HU) may therefore be powerful and affordable supplements to HIV-1 antiretroviral therapy.

Clinical trials

Recent clinical trials in HIV-1-infected patients have investigated the long-term synergistic effect of HU on conventional antiretroviral therapy with the nucleoside analogue didanosine (ddI). Vila *et al.* [1] treated ddI-naive individuals with CD4 cell counts above 200×10^6/l with HU and ddI, and reported that after 1 year, 10 out of 20 patients had no detectable virus in plasma or lymphoid tissue. Two of these patients stopped therapy and had extracellular virus remain undetectable in both lymph nodes and plasma for 1 year [2]. Similarly, Lori *et al.* [3] reported that after 72 weeks of ddI–HU treatment, three out of six patients had no detectable plasma virus, and that there was no rebound of the plasma viral load in any patient on uninterrupted treatment. There is also an intriguing anecdotal report of a patient on indinavir, ddI and HU, who after having had HIV driven down to an undetectable level stopped taking these drugs, and remained undetectable for 9 months [4].

Short-term studies report similar encouraging results of the ddI–HU combination in patients naive for ddI. During the first month of treatment the viral load decreases sharply by 1–2 $\log_{10}$ copies/ml, and several patients had undetectable virus levels after 3 months [5], 4 months [6], or 6 months [7] of treatment. In another study, 1000 mg daily HU treatment added to chronic ddI therapy decreased viral load by approximately 1 $\log_{10}$ copies/ml and decreased CD4 cell count by 25% [8]. The combination of HU with ddI is more potent than combinations with other nucleoside analogues [9], probably because HU preferentially depletes intracellular dATP concentrations [10,11]. However, monotherapy with HU failed to have a beneficial effect on plasma HIV RNA load (but may decrease CD4 cell count) [12,13]. Two studies have compared ddI monotherapy with the ddI–HU combination. They either failed to find a difference [14], or reported a significantly stronger decrease in plasma viraemia with the

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ddI–HU combination [7]. Patients on ddI–HU treatment routinely develop mutations known to confer resistance to ddI [7,11]. However, since ddI-resistant mutants grow poorly in the presence of HU [11], these patients have a lower plasma virus concentration than those on ddI monotherapy [7,11].

Efficacy of ddI–HU

Why is long-term treatment with ddI–HU effective? HU blocks the cellular enzyme ribonucleotide reductase, which thus decreases the intracellular concentrations of nucleosides required for DNA synthesis [9,15]. By decreasing the intracellular dATP pool, HU may favour the incorporation of ddI [11]. HU is also a cell cycle-specific toxin [16] that inhibits the S-phase of the cell cycle [17]. It is a routinely prescribed cytostatic drug used in the treatment of leukaemia [18] and to kill dividing T cells [19,20]. Although the HU dosage employed in ddI–HU trials should be low enough to avoid haematological side-effects [18], most (but not all [5]) of the HU trials in HIV patients report suppression [8,12,13] or poor recovery [1,3,6] of peripheral blood CD4 cell counts. This negative impact on peripheral blood CD4 cell counts is an important difference between the ddI–HU combination and other forms of antiretroviral therapy. By killing dividing CD4+ T cells and by depleting intracellular dATP concentrations HU reduces the availability of suitable target cells for HIV. Using mathematical models we have shown that such a reduction of target cell availability during antiretroviral treatment can strongly reduce the growth rates of drug-resistant escape mutants [21]. This effect, we believe, explains the encouraging long-term effects of the ddI–HU combination [1–3], even in the apparent presence of ddI-resistance mutations [7,11].

There is ample evidence that the availability of activated CD4+ T cells limits HIV-1 levels during clinical latency. Stimulating the immune system with interleukin-2 in the absence of potent antiretroviral therapy may increase the viral load [22]. Immunization of HIV-1-infected patients with either influenza vaccine [23,24], hepatitis B vaccine [25], pneumococcal vaccine [26], or tetanus toxoid [27], which should all activate T cells, tends to increase the viral load. A similar increase in HIV levels is seen during infection with pathogenic organisms [28,29]. The early rebound of wild-type virus observed during zidovudine treatment [30] finds a straightforward explanation in the increased target cell availability when the CD4 cell counts recover [30–33]. Finally, the high CD4+ T-cell production in children [34] may explain the high viral loads that HIV-infected children tend to have [35,36]. If HIV is target-cell-limited during clinical latency [37,38], one should be able to exploit this by immunosuppressive therapies decreasing target cell levels. Suppression with cyclosporine [39–42], prednisolone [43,44] and HU [1,3,5,6,8] indeed have beneficial effects.

Mathematical models

Analysing mathematical models in which the HIV infection is target-cell-limited one finds that for any strain of HIV there exists a minimum target cell number below which the strain cannot be maintained [21]. This threshold number is set by various viral characteristics, such as its infection rate, burst size, and lifespan [21]. This finding is identical to classical results in epidemiology stating that any infectious disease has a critical host density below which the infection cannot maintain itself. Because HIV-1 infection is at quasi-steady state during clinical latency [45,46], the steady-state target cell level should be close to this epidemiological threshold. Target cell numbers higher than this would allow a target-cell-limited virus to expand, which is consistent with the data reviewed above, while target cell numbers below this threshold will lead to viral decay [21,47].

Analysing antiretroviral therapy in the same mathematical model, we have predicted precisely the long-term effects that are observed now with the ddI–HU combination: the major beneficial effect of supplementing antiretroviral therapy with target cell suppression should be a reduced expansion of drug-resistant mutants [21]. Pre-existing drug-resistant variants, having a lower fitness than the pretreatment wild-type virus [48,49], require higher target cell levels than the wild-type virus in order to expand. Likewise, novel mutants arising under drug pressure are unlikely to attain a fitness higher than that of wild-type virus before the onset of treatment. Thus, the recovery of the CD4+ target cell population seems the 'Achilles heel' of conventional antiretroviral therapy: the increased target cell availability allows drug-resistant mutants to escape [21,30,33,47]. The encouraging long-term effect of ddI–HU treatment on the viral load, allowing in most cases only for a limited CD4 cell recovery, is therefore in good agreement with our conjecture that HU decreases target cell availability and consequently reduces, or even prevents, the outgrowth of drug resistant escape mutants [21].

Conclusion

Importantly, our results suggest that similar long-term beneficial effects are to be expected from the combination of HU, or other immunosuppressive agents, with other antiretroviral drugs. Obviously this should be

tested carefully because lowering CD4+ T cells may put patients at risk of even more opportunistic infections, and because immunosuppression would be harmful if the HIV infection is largely controlled by immune responses rather than by target cell availability. The current encouraging results with the ddI–HU combination nevertheless supports our conjecture that some degree of target cell depletion could be very beneficial by preventing drug resistance [21]. If this turns out to be true, it would open up inexpensive and well-tolerated new therapeutic strategies for patients not responding to current therapies, and for countries unable to afford them.

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