Effects of nucleoside-based antiretroviral chemotherapy on human T cell leukaemia/lymphotropic virus type 1 (HTLV-1) infection in vitro

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Human T cell leukaemia/lymphotropic virus type 1 (HTLV-1) infection is nowadays considered a global epidemic since 10–20 million individuals are estimated to be carriers of the virus and the risk of developing disease in endemic areas has been evaluated as 5% in asymptomatic patients. HTLV-1 is endemic in south-western Japan, in the Caribbean basin, where 3–4% of the population is seropositive for the virus, in North and South America and in some areas of Africa. The main modalities of transmission are: (i) perinatally, mainly breast feeding; (ii) parenterally, blood transfusion and sharing of needles and syringes infected with contaminated blood in drug abusers; and (iii) sexual contacts.¹

HTLV-1 is associated with a variety of clinical diseases. Initially it was linked to a chronic adult T cell leukaemia/lymphoma (ATLL), and, later, to HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP), both found in endemic areas of Japan and of Central and South America. ATLL is usually classified in five stages: asymptomatic, pre-leukaemic, chronic/smouldering, lymphoma and acute. Chronic/smouldering ATLL displays mild symptoms, whereas acute ATLL shows a rapid progression of the disease. Chronic ATLL presents a high number of leukaemic cells in the periphery whereas acute ATLL is characterized by lymphohadenopathy, hypercalcaemia and hepatosplenomegaly. Patients with HAM/TSP present spasticity of the lower extremities, weakness of the lower muscles and dysfunction of the urinary bladder.²³ Recently, HTLV-1 has also been associated with polymyositis, arthropathy and uveitis.¹

Anti-cancer chemotherapy has always been the only treatment for ATLL.⁴ In addition, combinations of classical anti-cancer chemotherapy with interferons (IFNs) and with monoclonal antibodies against the interleukin-2 (IL-2) receptor have been found to induce a limited benefit in 50% of ATLL patients. However, recently it has been reported that therapy with nucleoside-based antiretroviral agents, either alone or in combination with IFN, has the ability to control the proliferation of HTLV-1-infected cells and to assure a partial response in HTLV-1-infected patients.⁵⁸ Nevertheless, the use of nucleoside analogues in HTLV-1 therapy is still subjected to criticism because HTLV-1 infection in vivo is accompanied by limited viraemia. Furthermore, several in vitro studies have shown that the use of β-IFN or combinations of cytotoxic and antiproliferative drugs provides protection against virus transmission to cord blood CD4 lymphocytes⁹ or cell cycle arrest and apoptosis in HTLV-1 transformed cells,¹⁰ respectively. Conversely, not enough in vitro data were available to encourage the use of nucleoside-based antiretroviral therapy in HTLV-1-associated diseases. Thus, we focused our attention on this aspect. A possible, disease-related method to investigate the potentialities of nucleoside analogues in HTLV-1 infection is to analyse the effects of the drugs on virus transmission using reproducible in vitro models.

**HTLV-1 infection in vitro**

HTLV-1 is a transforming retrovirus, which can infect a broad range of lymphoid and non-lymphoid cells in vitro, although it preferentially immortalizes CD4 cells. HTLV-1 is a highly cell-associated virus. Thus, it is transmitted in vitro by cocultivation of the recipient cells with chronically infected, irradiated virus-donor cells¹¹,¹² more easily than by exposure to cell-free supernatant. HTLV-1-infected cells, kept in culture by addition of IL-2, in the long run might become immortalized by the virus. Using experimental models of infection in vitro it is possible to investigate the effect of antiretroviral drugs on HTLV-1 at an early stage of viral transmission and in the immortalization phase. A pioneer in vitro study to assess the protective effect of nucleoside analogues towards HTLV-1 infection in 1987, showed that the addition

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of 3′-azido-2′-3′-dideoxythymidine (zidovudine; AZT), at concentrations from 3 to 27 µM, protected antigen-specific human T cell clones from HTLV-1 infection. Protection was shown by inhibition of both proviral DNA integration and expression of core Gag proteins. In order to simulate a naturally occurring infection, in our studies on the efficacy of nucleoside analogues in HTLV-1 infection, we opted for fresh, peripheral blood mononuclear cells (PBMC) isolated from adult normal donors, either in toto or as a source for isolated CD4 cells, as recipient cells. In our experiments, the drugs were added at the onset of the co-culture and several times after, trying to reproduce a scheme of in vivo treatment. Other new, technical, aspects of our studies were that the effects of the drugs were tested on unstimulated cells and that drug concentrations used were also lower than 3 µM. Co-cultures were checked for viral presence after 3–4 weeks of infection, by analysing the presence of virus through DNA or RNA PCR and by evaluating the expression of the Tax protein. Such timing was chosen in order to exclude the presence of contaminating material from irradiated donor cells that could interfere with the molecular determination of the virus in de novo infected PBMC. In fact, repeated assays showed that irradiated cells alone did not express viral RNA or persist in culture for more than 2 weeks. Our studies showed that repeated treatment of PBMC with 8 to 32 µM zidovudine in vitro caused 90% inhibition of proviral DNA, as assayed by DNA PCR. Lower concentrations of zidovudine, such as 2 µM, were able to maintain infection at a low level, although protection was not complete and varied in different donors. Viral inhibition was detected at transcriptional as well as at translational levels, as shown by decreased RNA and protein expression. Inhibition of viral expression could very likely be considered as the consequence of a lower number of copies of integrated viral DNA. The effect of zidovudine was apparently due to its well known direct action towards reverse transcription, since pre- or post-treatment of target cells with zidovudine did not affect susceptibility to virus infection. However, one of the drawbacks of zidovudine is its anti-proliferative activity towards host cells. Particularly, zidovudine greatly inhibited proliferation of both infected and uninfected cells. Nevertheless, the 50% inhibitory concentration (IC50) value of the antiproliferative effect was 25 times higher than that of the antiviral effect, suggesting that the two events in infected cells represent phenomena attributable to distinct mechanisms. In addition, it might be considered that zidovudine, once incorporated as an abnormal nucleoside into the cellular DNA of treated PBMC, is genotoxic. In fact, a number of studies have shown that zidovudine induces extensive genomic deletion in treated cells. However, zidovudine was able to inhibit viral transmission to more immature, immune competent cells also at relatively low concentrations. In fact, cord blood mononuclear cells (CBMC), which are more sensitive than adult PBMC to HTLV-1 infection, were protected against infection at zidovudine concentrations as low as 0.03 µM. This suggests that it could be possible to inhibit HTLV-1 transmission from mother to child at zidovudine doses that should be of low toxicity or non-toxic for the fetus or for the newborn baby. Higher concentrations of zidovudine induced apoptosis in uninfected CBMC. This should generally be considered as the consequence for target cells to protect themselves from the ability of zidovudine to produce DNA damage. However, the exact mechanisms by which zidovudine causes apoptosis of target cells has not been elucidated. Thus, based on the intrinsic characteristics of zidovudine it could be hypothesized that the capability of zidovudine to protect HTLV-1-infected PBMC or CBMC from immortalization, in the long run, could involve not only its antiviral effect as reverse transcriptase inhibitor, but also its genotoxic/mutagenic property. Clinical investigations suggested that thia-2′-3′-dideoxyctydine (lamivudine; 3TC) could also be, at least partially, efficacious in the treatment of HAM/TSP or ATLL. However, recent in vitro studies have cleared up some misconceptions about using lamivudine in HTLV-1-infected individuals. In fact, we showed that lamivudine completely inhibited HTLV-1 infection of PBMC in vitro only at concentrations considered out of the range of those used in antiviral therapy. We have shown that, unlike anti-HIV activity, lamivudine was 100 times less active than zidovudine in inhibiting HTLV-1 transmission to PBMC, as determined by IC50 calculation. Unexpectedly, however, lamivudine at concentrations as low as 6.25 µM, i.e. within the therapeutic range, was able to counteract immortalization of infected cells. This effect might be due to mechanisms other than a direct, antiviral action, such as partial inhibition of transactivating Tax protein expression. Our observation on the limited antiviral potency of lamivudine in HTLV-1 infection in vitro is consistent with a biochemical study showing the poor ability of lamivudine to interact with HTLV-1 reverse transcriptase, as assessed through an enzymic assay. Anyhow, we have shown that lamivudine has a less toxic effect on immune cells than zidovudine in vitro, since it poorly inhibits proliferation of either infected or uninfected lymphocytes. Table 1 shows

<table>
<thead>
<tr>
<th>Drug</th>
<th>IC50 ± SD (µM)</th>
<th>CC50 ± SD (µM)</th>
<th>SI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lamivudine</td>
<td>14 ± 1</td>
<td>80 ± 27</td>
<td>5</td>
</tr>
<tr>
<td>Zidovudine</td>
<td>0.08 ± 0.01</td>
<td>6.6 ± 1.0</td>
<td>82</td>
</tr>
</tbody>
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*aInhibitory concentration 50%.
*bCytotoxic concentration 50%.
*cCC50/ID50 ratio.
*dDerived from data reported in reference 17.
*eDerived from data reported in reference 14.
the comparison between anti-HTLV-1 activity of lamivudine and zidovudine by reporting the selectivity index (SI) for each drug, as defined by the ratio of concentrations required to reduce cell growth by 50% (CC50) to IC50.

Conclusions

Studies undertaken to assess the activity of nucleoside analogues towards HTLV-1 infection should consider that this virus is associated with a chronic, low-productive infection, and that its immortalizing/transforming potentiality is due to its preferential integration within the host cell genome. These characteristics justify the preponderant modality for HTLV-1 to propagate infection in vivo by vertical more than by horizontal transmission. Therapy with nucleoside analogues in HTLV-1 infection might have a number of different implications. In fact, on one hand the objective to achieve is an optimal direct action against the retro-transcription of proviral DNA, similar to what occurs in anti-HIV therapy. On the other hand, the drug should exert, as much as possible, a specific cytotoxic effect towards infected cells, possibly by means of interaction with cell cycle regulating viral genes. Finally, since the chronicity of HTLV-1 infection leads us to predict a long-term treatment, the fact that cells of individuals under therapy could collect nucleoside-analogue induced mutations should be taken into consideration. These mutations could target either cellular structural genes or genes encoding repair enzymes, causing the death of infected cells even after a long time, or enhancing the mutagenic property of the virus itself. The existence of experimental models of long-term infection in vitro by HTLV-1 offers an extremely good opportunity to test the efficacy of nucleoside-based drugs as anti-HTLV-1 chemotherapeutic agents looking at all these aspects. Nevertheless, coordinated pre-clinical studies to assess the susceptibility of HTLV-1 to new nucleoside analogues are still lacking. Unfortunately, results obtained in HTLV-1-infected patients using nucleoside analogues utilized for HIV infection, are discouraging in that only a partial and temporary success in decreasing viral load has been achieved.8,9 This suggests that it could be worthwhile to seek new nucleoside analogues as potential anti-HTLV-1 chemotherapeutic agents. They should even be distinct from those used to treat HIV infection, with different substitutions of functional groups within the backbone structure of the molecule, in order to accomplish better control of both viral replication and host cell-to-cell viral transmission in HTLV-1-infected individuals.

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References


