Review

Intracellular Phosphorylation of Zidovudine (ZDV) and Other Nucleoside Reverse Transcriptase Inhibitors (RTI) Used for Human Immunodeficiency Virus (HIV) Infection

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Dramatic reductions of viral load and increased survival have been achieved in patients infected with the Human Immunodeficiency Virus (HIV) with the introduction of combination antiretroviral therapy. Currently 11 agents including nucleoside reverse transcriptase inhibitors (RTI), non-nucleoside RTI and protease inhibitors are available for the use for treatment of HIV infection. Recent studies have demonstrated that certain combinations of these drugs are advantageous over their individual use as monotherapy with an even more sustained viral suppression. Much emphasis has therefore been put on studies evaluating the interactions of these different compounds. Especially the intracellular metabolism of nucleoside RTI has been evaluated to some extent, by both in vitro and in vivo studies. These compounds need to undergo phosphorylation to their active 5'-triphosphates involving several enzymatic steps and the nucleoside concentration in the plasma may not correlate with intracellular concentrations of active drug. It is therefore of great importance to study these drugs at an intracellular level in order to evaluate their efficacy. This review summarizes the intracellular phosphorylation of Zidovudine and other nucleoside analogs investigated by in vitro experiments and the efforts of measuring the active anabolites in vivo in cells isolated from HIV infected patients on nucleoside therapy.

KEY WORDS: human immunodeficiency virus (HIV); AIDS; nucleoside therapy; nucleoside reverse transcriptase inhibitors; zidovudine; intracellular phosphorylation.

INTRODUCTION

The Acquired Immune Deficiency Syndrome (AIDS) is a degenerative disease of the immune system caused by the Human Immunodeficiency Virus (HIV), a lentivirus belonging to the family of the retroviridae (1–3). HIV infection causes a severe depletion of CD4 expressing cells which include T lymphocytes, monocytes and macrophages leading to a profound immuno suppression. Considerable progress has been achieved during the last decade in understanding of viral RNA and DNA kinetics and immune responses during primary and clinically latent phases of HIV infection. For example, sensitive PCR methods have revealed that HIV RNA can be detected in plasma at any stage of the disease (4–6). In addition, the lymphoid tissue has been shown to be a major site of virus replication with virus concentrations in some asymptomatic individuals that are approximately 1 to 2 log units higher than in the peripheral blood (7,8).

Dramatic reductions of viral load have recently been achieved by the introduction of combination antiretroviral drug therapy. Currently three classes of drugs including 11 agents are in use for HIV infection (Table 1) (9). These classes include the nucleoside reverse transcriptase inhibitors (RTI), non-nucleoside RTI and the more recently introduced protease inhibitors. The RTI specifically inhibit the transcription of viral RNA to proviral DNA whereas the protease inhibitors inhibit the cleavage of precursor proteins into mature functional viral proteins, which is essential for infectivity of the virus (Figure 1).

Zidovudine (ZDV), the first compound that has been approved by the FDA, was long used as mono therapy for HIV infection. However, recent studies have demonstrated that the combination of ZDV with other available compounds leads to a more sustained viral suppression. For example the use of two nucleoside RT inhibitors ZDV plus either didanosine (ddI), zalcitabine (ddC) or lamivudine (3TC) has demonstrated a sustained reduction of plasma viremia with an increased CD4 count, which have shown clear clinical benefits. [Similar effects have been obtained with the use of ddI plus stavudine (d4T), ddI plus 3TC, and d4T plus 3TC (9)]. Promising results have also been seen with triple RTI therapy including dDI, d4T and 3TC. Studies with two RT inhibitors in combination with either a protease inhibitor or a non-nucleoside RT inhibitor have shown that viral replication can be suppressed to less than detectable levels in previously untreated subjects (9). These new methods of treatment are allowing new hope for HIV infected patients although questions as to when to initiate ther-

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Table 1. Antiretroviral Drugs in Combination Therapy of Human Immunodeficiency Virus (HIV) Infection

<table>
<thead>
<tr>
<th>Nucleoside reverse transcriptase inhibitors</th>
<th>Protease inhibitors</th>
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<tbody>
<tr>
<td>Zidovudine (3'-Azido-3'-dideoxythymidine, AZT, ZDV)</td>
<td>Nevirapine</td>
</tr>
<tr>
<td>Stavudine (3'-Deoxy-2',3'-dideoxythymidine, d4T)</td>
<td>Delavirdine</td>
</tr>
<tr>
<td>Zalcitabine (2',3'-Dideoxyctydine, ddC)</td>
<td>Didanosine (2',3'-Dideoxyinosine, ddl)</td>
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<tr>
<td>Lamivudine (2',3'-Deoxy-3'-thiacytidine, 3TC)</td>
<td>Non-nucleoside reverse transcriptase inhibitors</td>
</tr>
<tr>
<td>Didanosine (2',3'-Dideoxyinosine, ddl)</td>
<td>Indinavir</td>
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therapy, which agents to use first, and how to adjust and when to change therapy, remain to be answered.

Much emphasis has been put on the evaluation of intracellular metabolism of the nucleoside RTI, since they are required to undergo activation to their active triphosphates inside target cells. Because host cellular kinases catalyze the phosphorylation to the active compounds, the RTI may compete for these enzymes and may therefore influence each other’s metabolism and subsequently their antiviral activity. Many studies have therefore been conducted to investigate the intracellular phosphorylation of these compounds in vitro and in vivo. This review summarizes in particular the findings for ZDV as well as for other nucleoside analogs obtained recently.

**PHOSPHORYLATION OF NUCLEOSIDE ANALOGS**

The viral RT is essential for the transcription of viral RNA to proviral DNA in the cytoplasm. Although the 2',3'-dideoxynucleosides (ddN) have distinguished affinities to the human α, β and γ DNA polymerase, resulting in different safety profiles, they have a much higher affinity for the viral RT, which makes the latter an excellent target for specific inhibition of the viral replication. However, 2',3'-dideoxynucleoside analogs as shown in Figure 2 have no intrinsic activity. In order to be active against HIV they must first enter the target cells and be phosphorylated by host cellular kinases to their active 5'-triphosphates (10–12). The various nucleoside analogs are phosphorylated by different enzyme systems to the mono- and di- phosphate, respectively (Figure 3). The last phosphorylation step to the triphosphate, however, is most probably catalyzed by the common nucleoside diphosphate kinase, an enzyme that can use purine or pyrimidine derivatives as substrate. The active triphosphates then competitively and potently inhibit the binding of endogenous nucleoside triphosphates to the viral RT. Once incorporated into the DNA chain, the nucleoside triphosphates act as chain terminator due to the absence of the 3'-OH group, which prohibits the 5' to 3' linkage that is required for chain elongation.

Plasma or serum pharmacokinetics as well as interactions with other drugs at a plasma level have been well established using methods such as High Performance Liquid Chromatography (HPLC) or a more sensitive radiomunnoassay method (RIA) (13). However, since all nucleoside analogs need to be phosphorylated to their active triphosphates involving several enzymatic steps inside the cell, extracellular concentrations

Fig. 1. Replicative cycle of the human immunodeficiency virus (HIV). 1 HIV attaches to the CD4 receptor and to a secondary receptor on the target cell via the viral envelope protein gp120. 2 HIV fuses with the cell membrane involving the protein gp41. The viral capsid gets subsequently uncoated and the viral RNA is released into the cytoplasm. 3 The transcription of the viral RNA into proviral DNA is catalyzed by the viral reverse transcriptase (RT). 4 After duplication of the single strand, the viral RNA is integrated into the human genome by the viral integrase. 5 The replication of viral DNA is followed by transcription of proviral DNA into mRNA. 6 The mRNA is then translated into proviral proteins, which need to undergo further maturation such as cleavage or glycosylation. 7 In a last step the proteins are assembled and the virus is budding through the cell membrane.

Fig. 2. Structure of 2',3'-dideoxynucleoside analogs currently in clinical use. 3'-Azido-2',3'-dideoxythymidine (AZT, Zidovudine); 2',3'-dideoxy-2',3'-dideoxythymidine (d4T, Stavudine); 2',3'-dideoxyinosine (ddl, Didanosine); 2',3'-dideoxyctydine (ddC, Zalcitabine); (−) 2'-deoxy-3'-thiacytidine (3TC, Lamivudine).