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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′-triphoshates 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 ^{10}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 nucluoside RT inhibitors ZDV plus either didanosine (ddI), zalcitabine (ddC) or lamuvidine (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

Nucleoside reverse transcriptase inhibitors
Zidovudine (3′-Azido-3′-dideoxythymidine, AZT,ZDV)
Stavudine (3′-Deoxy-2′,3′-didehydrothymidine, d4T)
Zalcitabine (2′,3′-Deoxycytidine, ddC)
Lamivudine (2′,3′-Deoxy-3′-thiacytidine, 3TC)
Didanosine (2′,3′-Dideoxyinosine, ddI)
Non-nucleoside reverse transcritase inhibitors
Nevirapine
Delavirdine
Protease inhibitors
Indinavir
Saquinavir
Ritonavir
Nelfinavir

apy, 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.

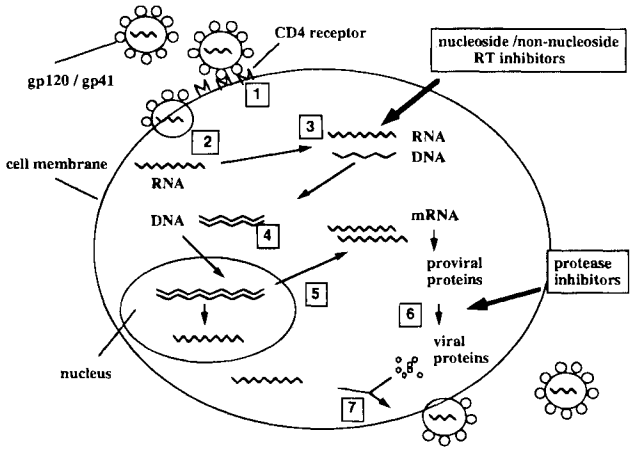

Fig. 1. Replicative cycle of the human immunodeficiency virus (HIV). **1** HIV attaches to the CD4 receptor and to a secondary receptor on the target cell via the viral envelope protein gp120. **2** HIV fuses with the cell membrane involving the protein gp41. The viral capsid gets subsequently uncoated 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 DNA 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.

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 radioimmunoassay 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

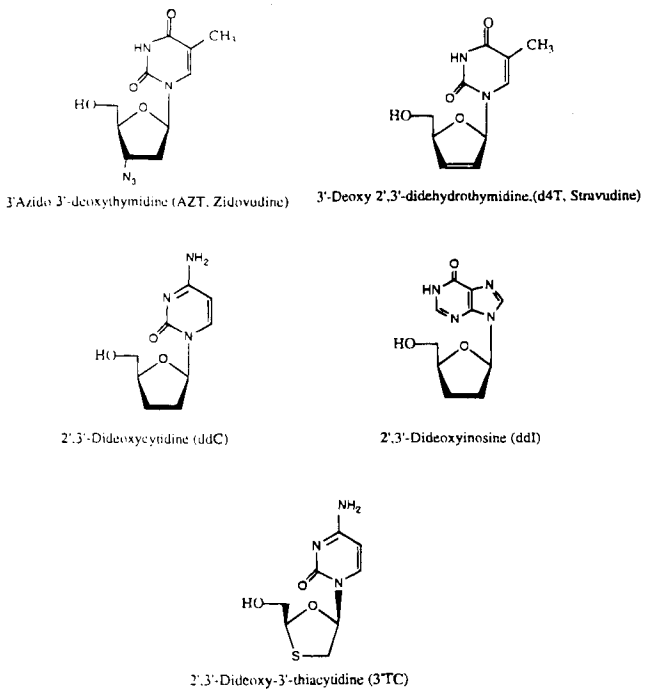

Fig. 2. Structure of 2′,3′-dideoxynucleoside analogs currently in clinical use. 3′-azido-2′,3′-deoxythymidine (AZT, Zidovudine, ZDV); 2′,3′-didehydro-2′,3′-dideoxythymidine (d4T, Stavudine); 2′,3′-dideoxyinosine (ddI, Didanosine); 2′,3′-dideoxycytidine (ddC, Zalcitabine); (−) 2′-deoxy-3′-thiacytidine (3TC, Lamuvidine).

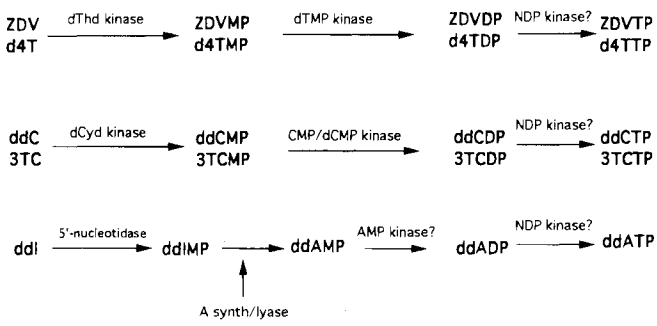

Fig. 3. Phosphorylation pathways of parent nucleoside analogs to their active triphosphates. **MP:** monophosphate; **DP:** diphosphate; **TP:** triphosphate; **dThd:** deoxythymidine; **dTMP:** deoxythymidine monophosphate; **NDP:** Nucleoside diphosphate; **dCyd:** deoxycytidine; **CMP:** Cytidine monophosphate; **AMP:** adenosine monophosphate; **A synth/lyase:** adenylosuccinate synthetase/lyase.

of the parent nucleoside may not necessarily correlate with intracellular concentrations of active drug. In addition, it has been shown in the case of ZDV that certain patients have difficulties in phosphorylating this compound at all (Sommadossi et al., personal communication). It is therefore of great importance to investigate the metabolism and interaction of these individual compounds inside the target cells in order to predict efficacy of the nucleoside analogs at the site of activity.

PHOSPHORYLATION OF INDIVIDUAL NUCLEOSIDES

Since ZDV has been the first compound to be used for HIV infection, its intracellular metabolism has been thoroughly studied. ZDV is an analog of thymidine and permeates the cell membrane by non-facilitated diffusion (14). In fact, the uptake of ZDV by target cells is insensitive to nucleoside transport inhibitors such as dipyridamol or nitrobenzylthioinosine and is also insensitive to an excess of natural nucleosides. The first phosphorylation step to the monophosphate is catalyzed by the cytosolic thymidine kinase. ZDV as well as endogenous thymidine have a comparable affinity for the thymidine kinase with Km values of 3.0μmol/L and 2.9mmol/L, respectively (10–12). The Vmax of ZDV for the thymidine kinase is 60% of that of the natural substrate. Thymidilate kinase and nucleoside diphosphate kinase phosphorylate the monophosphate further to the di- and triphosphate, respectively (10–12). The apparent Km value of the thymidilate kinase is two times greater for zidovudine monophosphate than that for deoxythymidin monophosphate (dTMP) (8.6μmol/L versus 4.1μmol/L). However, the Vmax is only 0.3% of that of dTMP. As a result ZDV-monophosphate accumulates inside the cell accounting for about 95 to 99% of the total phosphates. On the other hand, the di- and active triphosphate are present in equal concentrations, accounting for the remaining balance (11,12). Stavudine (d4T) is another thymidine analog that is phosphorylated by the same enzymatic system as ZDV. The mono-, di- and triphosphate ratios of these two drugs, however, are considerably different in human lymphatic cell lines (15). These findings are due to the fact that the rate limiting enzymes are different for both compounds: thymidine kinase for d4T and thymidilate kinase for ZDV. This is confirmed by experiments demonstrating that an increase of the extracellular d4T concentration increases the total intracellular phosphates by about 120 fold, whereas the ratios of mono- di and triphosphate remain the same. On the other hand an increase of extracellular ZDV by more than 1000 fold raises the monophosphate concentration 150 fold, whereas the di- and triphosphate increase only about 4-fold. In comparison, the unphosphorylated d4T inside the cell accounts for approximately 47% and the triphosphate for 34%. D4T-mono- and diphosphate are present in approximately equal concentrations (11,12,15). Since ZDV and d4T share the same enzymatic system for their phosphorylation, one might expect an interaction of the two drugs at the cellular level when administred in combination. Indeed, Hoggard et al. (16) have demonstrated by *in vitro* studies that the incubation of PBMC with both drugs at therapeutically achievable plasma concentrations resulted in a reduction of intracellular phosphate metabolites of d4T. On the other hand, ZDV phosphorylation remained relatively unaffected. These findings are consistent with data suggesting that thymidine kinase has a 600-fold lower affinity for d4T that for ZDV (15).

In contrast to ZDV and d4T, ddC and 3TC are cytidine derivatives. The phosphorylation of these compounds to their monophosphate is catalyzed by deoxycytidine kinase. Cytidine monophosphate kinase and nucleoside diphosphate kinase then phosphorylate the monophosphate further to the di- and triphosphate, respectively. ddC is a weak substrate for the cytidine kinase and only about 50% of the parent nucleoside gets further phosphorylated to mono-, di-, and triphosphate in an HIV sensitive T-cell line (OKT4+, ATH8) (17). For example, the Km values in Molt-4 cells and peripheral lymphocytic leukemia cells are 200 and 180 μmol/L, respectively. While ddC-triphosphate accounts only for about 10% of the total phosphates, the percentage of 3TC-triphosphate was found to be approximately 40% of total phosphates in stimulated, HIV infected peripheral lymphocytes, when the respective nucleosides were tested (18). Similar to the thymidine analogs ZDV and d4T, ddC and 3TC share the same enzymatic system for intracellular phosphorylation. It is therefore not surprising that Veal et al. (19) demonstrated that 3TC significantly inhibited ddC phosphorylation in PBMC, when incubated with both drugs simultaneously.

Studies of didanosine (ddI) metabolism have revealed that this compound exerts its antiviral activity by virtue of its ability to generate dideoxyadenosine triphosphate (ddATP). An initial step of phosphorylation of ddI to ddIMP is known to be catalyzed by cytosolic 5'-nucleotidase followed by amination via adenylosuccinate synthetase/lyase enzymes to ddAMP. ddAMP seems to be further phosphorylated by adenylate kinases using ATP as a phosphate donor. However, the phosphorylation of ddAMP to ddATP requires further investigation (20–22). Analyzing the intracellular pool of ddI metabolites in three different cell lines revealed that only about 3% of the ddI is converted to ddATP.

The phosphorylation of the different nucleoside analogs is highly dependent on the activation state of the target cells (23,24). For example, the activity of thymidine kinase is regulated in a cell cycle dependent manner, and it is therefore not surprising that the nucleosides ZDV and d4T are preferentially phosphorylated in activated cells. But contrary to predictions from published in vitro studies, ZDV is also found to be a potent inhibitor of proviral DNA synthesis in resting cells (25). The phosphorylation of ddC and 3TC is quite efficient in resting

cells, but is even further enhanced in activated cells. Interestingly, however, both compounds appear to be more potent in resting cells. The enzyme system responsible for ddI activation on the other hand is not affected by the cell cycle and the levels of phosphorylation seems to be equal in activated and resting cells (23,24).

The most important factor for inhibition of HIV is the interaction of the 2′,3′-dideoxynucleoside-5′-triphosphates with the endogenous 2′-deoxynucleoside-5′-triphosphate compounds and the resulting ratios (ddNTP/dNTP) from these interactions, since they directly compete with each other for the viral reverse transcriptase (23,24). *In vitro* studies using different cell types have shown that ZDV decreases the thymidine triphosphate pool substantially, in H9 cells up to 4-fold. However, this decrease depends strongly on the cell type. d4T on the other hand decreases the thymidine triphosphate pool only moderately. Overall, the ratios of ZDVTP/TTP and d4TTP/TTP are higher in activated cells, which may explain the better antiviral activity of ZDV in these cells (23,24). Exposure to ddC resulted in a profound depletion of the cellular dCTP pool, especially in resting cells. In comparison, ddI and 3TC had no significant effect on any of the intracellular nucleosides. However, 3TC, ddC and ddI produced higher ratios in resting cells, which may explain why ddC and 3TC are more potent in resting cells, although their phosphorylation is enhanced in dividing cells. In regard to the ddNTP/dNTP ratios, Hydroxyurea (HU), a compound that has been approved for the treatment of oncological diseases, has recently gained much attention. HU is an inhibitor of the ribonucleotide reductase, the enzyme responsible for the conversion of the four ribonucleotides to deoxyribonucleotides, a process that is essential for DNA synthesis (26). Since HU can selectively inhibit DNA synthesis it has been evaluated for its capability to inhibit HIV-1 replication. Furthermore, the possiblity of potentiating the activity of nucleoside analogs has been evaluated, since the endogenous intracellular dNTP pool may be decreased after administration of HU. Indeed, it was found that HU not only inhibited virus replication but also potentiated the activity of ddI as well as that of ZDV and ddC, although to a lesser extent (26,27).

In conclusion, the phosphorylation of 2′,3′-dideoxynucleosides and their effect on endogenous nucleotide pools seems to be as important as the interaction with the viral reverse transcriptase. This has been confirmed by Hao et al. (28), who showed that differences in the ability of the dideoxynucleosides to generate their corresponding 5′-triphosphate correlates strongly with their antiviral activity in lymphoid cells. Furthermore, the nucleoside analog 2′,3′ dideoxyuridine (ddU) shows no inhibition of the viral replication, because of the failure to be efficiently anabolised to the 5′-triphosphate. The ddU-triphosphate, however, proved to be a potent inhibitor of the viral RT *in vitro* (29).

HIV replicates more efficiently and more rapidly in dividing cells. However, proviral DNA synthesis in resting cells seems to be initiated as efficiently as in activated cells (30–32). To ensure optimal combination therapy in patients, the available approved drugs should preferably be studied in combination, since intracellular interactions may increase or diminish antiretroviral activity. For instance, the combination of ZDV and d4T could be recommended based on monotherapy studies, whereas combination studies have revealed that ZDV reduced the phosphorylation of d4T to a large extent.

IN VIVO MEASUREMENT OF INTRACELLULAR NUCLEOTIDES

The phosphorylation patterns and activity of the individual nucleoside analogs have primarily been analyzed by *in vitro* studies using radioactive substances in different cell lines or in peripheral lymphocytes isolated from HIV infected patients or seronegative volunteers. However, enzyme systems may become altered in culture and may therefore not necessarily reflect the *in vivo* situation. Many efforts have consequently been made to measure intracellular nucleotide concentrations directly in peripheral blood mononuclear cells (PBMCs) isolated from patients on antiretroviral therapy. Since radioactive compounds cannot be used in patients, novel methodologies needed to be developed to measure the phosphorylated anabolites inside the cells. Because ZDV has been in clinical use for many years, it has been used as a model compound for the development of an assay for the intracellular measurement of its mono-, di- and triphosphate.

Concentrations of ZDV nucleotides are extremely low and impossible to directly detect with standard UV, fluorescence or electrochemical detectors after high performance liquid chromatography (HPLC) or any other separation method. More sensitive approaches, such as bioassays are therefore needed to detect these low levels. Toyoshima et al. (33) were one of the first to report an analytical method for the measurement of intracellular ZDV nucleotides without using radiolabeled compounds. They employed an HPLC system using a column switching technique between two columns in which the first was a gel chromatography column for preseparation of ZDV metabolites from major cell components through differences in molecular size of the solutes. The actual ZDV metabolites were then separated and quantitated using an ODS column with a mobile phase consisting of potassium phosphate buffer, acetonitrile and dodecyltriethylammonium salt as an ionpairing agent.

Several other investigators (34–37) published methods using a combination of HPLC and radioimmunoassay. Using this method, the individual ZDV nucleotides are separated by HPLC in a first step. The fractions collected during this separation are cleaned by solid phase extraction to eliminate components from the mobile phase, which could interfere with the radioimmunoassay. Finally, the individual nucleotides are dephosphorylated by alkaline- or acid phosphatase to ZDV and assayed by a commercially available radioimmunoassay method (INCSTAR, Stillwater, MN, USA). The latter step is necessary, since the antibody used in the radioimmunoassay is only specific for the parent ZDV. Major differences in these assays can be found in the HPLC separations. Kuster et al. (34) and Slusher et al. (36) used ionexchange columns, whereas Stretcher et al. (35) and Peter et al. (37) utilized ODS columns, which required the ionpairing agent tetrabutyl ammonium phosphate (TBAP). Methodologies involving HPLC separations are unfortunately very time consuming, and can therefore not be used in routine analysis in clinical laboratories. Also, due to the low concentrations of ZDV-DP and ZDV-TP large blood samples (approximately 20 to 30 ml) are required to isolate sufficient numbers of cells. Stretcher et al. (38) therefore performed measurements of the total phosphorylated ZDV, which eliminated the time consuming HPLC separation. The extracted ZDV nucleotides were divided into two fractions, one of which was treated with alkaline phosphatase. Each fraction was then assayed by RIA

for ZDV and concentrations of phosphorylated ZDV determined by subtracting the concentration of the individual fractions from each other. This assay assumes that ZDV-monophosphate gets proportionally transformed into di- and triphosphate. However, as demonstrated by measurements in patients using the combination of HPLC-RIA, it has been shown that ZDV-MP does not correlate with ZDV-DP or ZDV-TP concentrations. Therefore, no predictions can be made for the active ZDV-TP by measuring the total phosphorylated compounds.

In an effort to simplify the quantitation of ZDV nucleotides, Robbins et al. (39) described an enzymatic assay for ZDV-TP based on the inhibition of the reverse transcriptase using purified HIV-1 RT and poly (rA)-oligo (dT) as template. This assay is more sensitive, and consequently the size of blood samples could be significantly reduced to approximately 10 to 20 ml. One drawback associated with this bioassay is the inability to differentiate ZDV-TP from endogenous nucleotides, and the standard curve was therefore performed in patients own ZDV free PBMC extracts. However, the assay assumes that the endogenous nucleotide concentrations remain the same after ZDV administration. Furthermore, the ZDV-MP, which may be partially responsible for toxicity of ZDV (40,41) cannot be determined by this methodology. A major improvment has been achieved by the same group using a combined catridge-radioimmunoassay methodology (42). With this approach, the HPLC separation of the individual nuleotides could be replaced by Sep-Pak cartridges, which reduces the time considerably, since several samples can be separated simultaneously.

Intracellular concentrations *in vivo* obtained using either of the above described methods were very similar and ranged from approximately 0.1 to 3pmol/10^6 cells for monophosphate and approximately 0.001–0.200 pmol/10^6 cells for di-, and triphosphate. Toyoshima et al. (33) on the other hand, measured much higher concentrations, which is probably due to differences in the methodology. The results obtained from these measurements also confirmed *in vitro* data, showing that there is a marked accumulation of ZDV-MP inside the cell, whereas only low levels of di-, and triphosphate derivatives are achieved.

Only a few clinical studies have been conducted so far measuring the intracellular phosphates over a time course. These studies have demonstrated that plasma ZDV concentrations do not correlate with any of the intracellular nucleotide concentrations, which emphasizes the importance of measuring these compounds inside the cell (37,43,44) In particular, Barry et al. (43) investigated the intracellular metabolism of mono-, -di-, and triphosphate in PBMCs in HIV infected patients and in seronegative volunteers. The conclusions from this study were that intracellular phosphorylation was higher in HIV infected individuals than in healthy volunteers. The concentration of ZDV-TP was similar in both groups and the increase in total phosphates was primarily due to an increase in ZDV-MP. This study also demonstrated a substantial increase in phosphorylation to ZDV-MP in patients with CD4 counts below 200 cells/mm^3. This may explain the increased toxicity found in this patient population, because ZDV-MP is at least partially associated with toxicity. Another study performed by the same group (45) investigated the effect of ZDV dose on the formation of intracellular phosphorylated metabolites. The investigator's hypothesis was that they may be able to reduce the ZDV dose without decreasing the efficacy. This hypothesis was based on *in vitro* studies showing that an extracellular increase of ZDV concentration leads only to a marked accumulation of ZDV-MP but not ZDV-DP or ZDV-TP. Using two different regimens (100mg three times daily and 300mg twice daily) they could indeed demonstrate that, with the lower dose only, ZDV-MP concentrations decreased significantly, but not the active TP, which may have a great impact on future dosing regimens. The effect of long term administration of ZDV on its intracellular phosphorylation in PBMCs was investigated by Peter et al. (44). Previous *in vitro* studies (46) and *in vivo* studies measuring the total phosphorylated ZDV (38) had implied a decrease of intracellular nucleotides over time. Mechanisms proposed for this decrease were a high expression of the multidrug transport protein, P-glycoprotein or mdr-1 gene product (multidrug resistance gene) or an impaired function of phosphorylating enzymes (46–50). In this study neither a change of intracellular phosphate concentrations nor an increase in P-glycoprotein expression could be found. Therefore, ZDV seems not to select for a higher P-glycoprotein expression in these cells. Due to the large blood samples required for the intracellular analysis, samples were only collected up to 6 hours in both studies. Although areas under the concentration-time curve could be calculated, which is an important measure for the amount present for HIV inhibition, the half-life of these nucleotides could not be accurately determined. Most recently intacellular ZDV nucleotide concentrations have been measured for the first time in mononuclear cells isolated from lymph nodes, and concentrations were compared to that in PBMC (Peter et al., submitted). This study was part of a larger multicenter study conducted by the Division of AIDS Treatment Research Group (DATRI 012). Interestingely the ZDV-MP concentrations in the lymph nodes were approximately 4 to 5 times greater than in the PBMCs. There may be several explanations for these results: 1) cells in the lymph node tissue may have a higher proliferation rate; 2) the cell population may be different leading to a different phosphorylation, since phosphorylation is cell type dependent; 3) ZDV concentration in this tissue may be higher leading to a higher intracellular ZDV concentration, which is then readily converted into ZDV-MP. The concentrations of ZDV-DP and ZDV-TP on the other hand were not significantly different in these two compartments. The exact reasons for this high concentration of ZDV-MP as well as its long term implication regarding toxicity remain to be elucidated. In conclusion, the different in vitro studies using radioactive labeled compounds, as well as the ability to measure ZDV nucleotides directly in cells isolated from patients on therapy, represent a significant advance in our understanding of the clinical pharmacology of nucleoside analogs used for HIV infection. However, many more questions need to be evaluated and more sensitive and faster methodologies need to be developed, not only for ZDV, but also for other nucleoside analogs. Much of this work is already under way in several laboratories.

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