Background: Alterations in endogenous nucleotide pools as a result of HIV therapy with nucleoside and nucleotide reverse transcriptase inhibitors (N[t]RTIs) is a proposed mechanism for therapy-related adverse events and drug interactions resulting in treatment failure. *In vitro* studies were performed in order to understand the effect of N[t]RTIs on endogenous nucleotide pools.

Methods: The T-cell line CEM-CCRF was treated with control antimetabolites or the N[t]RTIs abacavir, didanosine, lamivudine, tenofovir (TFV) and zidovudine (AZT), either alone or in combination. The levels of natural 2′-deoxynucleoside triphosphates (dNTP) and ribonucleoside triphosphates were determined by liquid chromatography coupled with triple quadrupole mass spectrometry.

Results: Antimetabolites altered nucleotide pools in a manner consistent with their known mechanisms of action. AZT was the only N[t]RTI that significantly altered dNTP pools. Incubation of 10 µM AZT, either alone or in combination with other N[t]RTIs, increased 2′-deoxyadenosine triphosphate, 2′-deoxyguanosine triphosphate and thymidine triphosphate levels by up to 1.44-fold the concentrations observed in untreated cells. At higher than pharmacological concentrations of AZT, evidence for inhibition of 2′-deoxycytidylate deaminase and enzymes involved in the salvage of thymidine was also observed. Phosphorylated metabolites of TFV are known to inhibit purine nucleoside phosphorylase (PNP). However, in contrast to a potent PNP inhibitor, TFV was unable to alter intracellular dNTP pools upon addition of exogenous 2′-deoxyguanosine.

Conclusions: N[t]RTIs have the potential to alter nucleotide pools; however, at the pharmacologically relevant concentrations, tested N[t]RTI or their combinations did not have an effect on nucleotide pools with the notable exception of AZT.

Nucleoside and nucleotide reverse transcriptase inhibitors (N[t]RTIs), used for the treatment of HIV infection, are activated by the cellular machinery responsible for regulating endogenous 2′-deoxynucleoside triphosphate (dNTP) and ribonucleoside triphosphate (rNTP) pools (summarized in Figure 1). Once formed, the active N[t]RTI triphosphate competes with its corresponding natural dNTP for incorporation by the viral reverse transcriptase or host DNA polymerases causing suppression of viral replication or toxicity, respectively. N[t]RTIs could cause perturbations in endogenous nucleotide metabolism by causing competitive inhibition, allosteric modulation or altered expression of nucleotide metabolizing enzymes (reviewed previously [1]). Due to the competitive nature of their incorporation by polymerases, changes in either N[t]RTI triphosphate levels or endogenous dNTP pools could alter the activity and toxicity of N[t]RTIs.

Treatment with zidovudine (AZT) is associated with adverse events including lipodystrophy and anaemia [2,3]. Different non-mutually exclusive mechanisms for toxicities associated with AZT therapy have been proposed including a decrease in intracellular thymidine salvage [4–6], direct inhibition of cellular polymerases through incorporation and chain termination [7,8], inhibition of 3′ to 5′ exonuclease proofreading by host DNA polymerases by AZT monophosphate (AZTMP) [9] and formation of a highly toxic metabolite through the metabolism of the 3′-azido group to an amine by cytochrome P450 and their reductases [10]. A direct role for AZTMP in toxicity observed in some cell types

Original article

Effect of nucleoside and nucleotide reverse transcriptase inhibitors of HIV on endogenous nucleotide pools

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has been suggested on the basis of the correlation of AZTMP levels with cytotoxicity in cultured cells [11]. AZT treatment might affect TTP levels by inhibition of cytosolic thymidine kinase (TK1), mitochondrial TK (TK2) and thymidylate kinase (TMPK). AZT and AZTMP have been observed to bind with similar affinity to TK1 and TMPK as their respective natural substrates, and the build-up of high levels of AZTMP might facilitate inhibition of thymidine phosphorylation [12].

Purine nucleoside phosphorylase (PNP) is a crucial enzyme in the regulation of purine nucleotide pools [13]. Results supporting inhibition of PNP by phosphorylated metabolites of tenofovir (TFV) as the mechanism for increased circulating levels of didanosine (ddl) when it is coadministered with TFV disoproxil fumarate (TDF, the oral prodrug of TFV) [14] led to the suggestion of further clinical implications for PNP inhibition [15]. Based on the effects of treatment

Figure 1. Pyrimidine and purine nucleotide metabolism pathways in mammalian cells

Schemes of (A) pyrimidine and (B) purine nucleotide metabolism pathways in mammalian cells (reviewed in a prior publication [28]). The enzymes adenine phosphoribosyltransferase (APRT), adenosine deaminase (ADA), 2'-deoxyadenosine deaminase (dADA), 2'-deoxycytidine deaminase (dDCA), 2'-deoxycytidine kinase (dCK), 2-deoxycytidylyl deaminase (dCMPDA), dihydrofolate reductase (DHFR), dihydroxymethylpyrimidine dehydrogenase (DD), hypoxanthine-guanine phosphoribosyltransferase (HGPRT), inosine monophosphate dehydrogenase (IMPDH), purine nucleoside phosphorylase (PNP), ribonucleotide reductase (RR), thymidine kinase (TK), thymidine phosphorylase (TP), thymidylate kinase (TMPK), thymidylate synthase (TS), uridine phosphorylase (UP) and xanthine oxidase (XOD) are shown. The nucleosides 2'-deoxycytosine (dC), 2'-deoxyguanosine (dG), 2'-deoxyuridine (dU), thymidine (T) and xanthine (X) are shown. Their mono-, di- and triphosphorylated forms are abbreviated as -MP, -DP and -TP, respectively.
with highly potent PNP inhibitors, including increased intracellular 2'-deoxyadenosine triphosphate (dATP) and 2'-deoxyguanosine triphosphate (dGTP) and inhibition of T-cell division [16], it was proposed that TFV might cause an increase in dNTP pools resulting in antagonism of the activity of other N(t)RTIs and the observation of decreased CD4+ T-cells with the combination of TDF and non-dose-adjusted ddi [17,18].

Triple N(t)RTI-only regimens including ddi/ lamivudine (3TC)/ stavudine (d4T), abacavir (ABC)/ ddi/d4T, ABC/3TC/AZT, ABC/ddI/TDF, ddi/3TC/TDF and ABC/3TC/TDF have shown high rates of virological non-response, viral rebound and resistance mutation selection [19–24]. Results from clinical studies with triple N(t)RTI-only regimens have led to a decision by a Department of Health and Human Services panel to not recommend therapy with ABC/3TC/AZT for treatment-naive patients and to state that other triple N(t)RTI-only regimens including ABC/3TC/TDF and ddi/3TC/TDF should not be considered at any time [25]. Different hypotheses have been proposed to explain the poor performance of triple N(t)RTI-only therapies including antagonism of intracellular N(t)RTI phosphorylation resulting in reduced triphosphate analogue levels, decreased activity of N(t)RTIs caused by increases in competing dNTP pools, an intrinsic lack of potency in N(t)RTI-only regimens, and the overlapping resistance profiles of N(t)RTIs included in these regimens.

In order to understand the effects of N(t)RTIs and their combinations on nucleotide pools, including the effect of AZT on thymidine phosphorylating enzymes and the inhibition of PNP by TFV, in vitro studies were carried out in CEM-CCRF cells. CEM-CCRF cells were chosen for their rapid cell division and high capacity for de novo and salvage nucleotide synthesis. A liquid chromatography coupled to tandem mass spectrometry method (LC/MS/MS) was developed using stable isotope-labelled nucleotides to allow for accurate and precise simultaneous analysis of the effects on both dNTP and rNTP endogenous pools.

Methods

Reagents

Cell culture supplies were purchased from Invitrogen (Carlsbad, CA, USA) and HyClone fetal bovine serum was purchased from Fisher Scientific (Pittsburgh, PA, USA). ABC, TFV and 3TC were supplied by Gilead Sciences Inc. (Foster City, CA, USA). AZT, 2'-deoxyadenosine, 2'-deoxyxanthosine, ddI, hydroxyurea, methotrexate and ribavirin were obtained from Sigma–Aldrich (St Louis, MO, USA). The trisodium salts of the natural dNTP and rNTP were also obtained from Sigma–Aldrich. Stable isotope-labelled [13C15N]thymidine and dNTP and rNTP were obtained from Spectra Stable Isotopes (Columbia, MD, USA). The PNP inhibitor 2-amino-7-[1-(2-fluorophenyl)-2-(R)-hydroxyethyl]-3,5-dihydro-4H-pyrrolo[3,2-d]pyrimidin-4-one (PNP405) was synthesized by NAEJA Pharmaceutical (Edmonton, AB, Canada) by methods similar to those previously reported [26].

Cells

Human T leukaemic CEM-CCRF cells were obtained from the American Type Culture Collection (Manassas, VA, USA) and were maintained in RPMI 1640 media supplemented with 10% heat-inactivated fetal bovine serum, 100 units/ml penicillin G and 100 µg/ml streptomycin sulfate.

Effect of antimetabolites and N(t)RTIs on nucleotide pools

CEM-CCRF cells were seeded at 0.5 million cells/ml in tissue culture flasks and treated for 24 h with the antimetabolites ribavirin, hydroxyurea or methotrexate at concentrations of 20 µM, 3 mM or 5 µM, respectively, or with N(t)RTIs either alone or in combination, each incubated at 10 µM.

Following the 24 h incubations, cells were counted using a haemocytometer and isolated from drug-containing media by spinning through a layer of oil as described previously [14]. After removal of the oil layer, cells were resuspended in 70% methanol and stored at -20ºC overnight to facilitate extraction of nucleotides. Cellular debris was then removed by centrifugation and the supernatant evaporated in a MiVac Duo concentrator (Genevac Inc., Valley Cottage, NY, USA). Samples were resuspended in 20 mM tetrabutylammonium acetate at a concentration of 1 million cells/10 µl for analysis by LC/MS/MS.

Effect of AZT and TFV on nucleotide salvage pathways

To study the concentration-dependent effects of AZT on the salvage of thymidine, 24 h incubations were done in the presence of 10 µM [13C15N]thymidine and 10, 100 and 1,000 µM AZT. The appearance of [13C15N]TTP was monitored as well as the levels of natural nucleotides. PNP inhibition-dependent changes in dNTP pools caused by 10 µM TFV relative to 10 µM PNP405 were studied following 24 h treatment of cells with the respective compound and 10 µM exogenous 2'-deoxyguanosine. Cells were prepared for analysis as described above.

Measurement of endogenous dNTP and rNTP levels by LC/MS/MS

Previously reported methods for the quantitative determination of intracellular dATP using standard curves of stable isotope-labelled dATP in cellular
matrices and ion-pairing reversed phase LC coupled to MS/MS detection [27] were extended to include all dNTP and rNTP. Analyses were performed using a CTC Analytics Leap autosampler (Leap Technologies, Carrboro, NC, USA), an LC-20AD tertiary pump LC system (Shimadzu Scientific Instruments, Columbia, MD, USA) and a Sciex API 4000 triple quadrupole mass spectrometer (Applied Biosystems/MDS Sciex, Foster City, CA, USA) running positive-ion and multiple reaction monitoring modes. Briefly, the natural and stable isotope-labelled nucleotide pairs were tuned for MS/MS detection by 10 µl/min infusions in 0.1 mM tetrabutyrammonium acetate and 20% acetonitrile to allow MS signal for each respective pair within 20% of one another. Parameters optimized included the mass to charge ratio of the parent and daughter ion, declustering potential, collision energy and collision exit potential. When necessary, a small correction factor was used to correct for differences in signals observed for the respective natural and stable isotope nucleotide pairs.

Standard curves of each stable isotope-labelled nucleotide were prepared in dried cellular extract reconstituted in 20 mM tetrabutyrammonium acetate and samples were separated using a multistage linear gradient of acetonitrile in a buffer containing 0.25 mM tetrabutyrammonium hydroxide and 4 mM ammonium phosphate on a 3 µm, 1.0×100 mm Luna C18 reverse phase column (Phenomenex, Torrance, CA, USA). The natural nucleotides in cellular samples were then quantitated by comparing their peak area to that observed for a standard curve of the corresponding stable isotope nucleotides. Nucleotide concentrations were reported in pmol/million cells on the basis of the determined amount of nucleotide in each injection of extract from 1 million cells.

Standard curves including seven concentrations and covering at least three orders of magnitude typically had linearity exceeding an r² value of 0.99. Lower limits of quantitation for 10 µl injections containing extracts from 1 million cells were approximately 20 fmol on the column for each nucleotide. To ensure accuracy and precision within 20% over the course of the analysis, a quality control sample containing natural and stable isotope-labelled nucleotides was injected at the beginning and end of each analytical batch.

Data analysis
All experiments were completed at least three independent times in duplicate or triplicate. The statistical significance of changes in endogenous nucleotide pools in treated relative to non-treated cells was evaluated using unpaired two-tailed Student’s t-tests.

Results
Measurement of dNTP and rNTP levels and the effect of known antimetabolites on nucleotide pools
The levels of dNTP (from 51.1 to 108 pmol/million cells) and rNTP (from 656 to 3,570 pmol/million cells) in untreated CEM-CCRF cells were similar to those reported in the literature for cultured tumour cell lines [28]. Studies were carried out with control antimetabolites to determine the sensitivity of CEM-CCRF cells to inhibition of nucleotide metabolizing enzymes (Table 1). Hydroxyurea, methotrexate and ribavirin are inhibitors of ribonucleotide reductase, dihydrofolate reductase and inosine monophosphate dehydrogenase (IMPDH), respectively (Figure 1). Consistent with their respective mechanisms of action, hydroxyurea treatment resulted in the depletion of all dNTP to 0.08–0.43-fold of the levels in untreated cells and methotrexate markedly reduced 2′-deoxyguanosine triphosphate (dTTP) and TTP to 0.08- and 0.12-fold of the levels, respectively, in untreated cells. No marked difference in cell number was observed following the 24 h incubations. General cytotoxicity was apparent in cells treated with these concentrations of hydroxurea and methotrexate by the decreases observed in almost all dNTP and rNTP pools. IMPDH

<table>
<thead>
<tr>
<th>Incubation</th>
<th>dNTP</th>
<th>rNTP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>dATP</td>
<td>GTP</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 mM Hydroxyurea</td>
<td>0.08 ±0.03</td>
<td>0.24 ±0.12</td>
</tr>
<tr>
<td>5 µM Methotrexate</td>
<td>0.47 ±0.06</td>
<td>0.08 ±0.01</td>
</tr>
<tr>
<td>20 µM Ribavirin</td>
<td>0.70 ±0.34</td>
<td>0.41 ±0.14</td>
</tr>
</tbody>
</table>

*Fold change in 2′-deoxyribonucleoside triphosphates (dNTP) or ribonucleoside triphosphates (rNTP) present in a side-by-side no treatment control (fold change = concentration in treated cells divided by concentration in untreated cells). Levels in untreated cells of 2′-deoxyadenosine triphosphate (dATP), 2′-deoxyguanosine triphosphate (dTTP), 2′-deoxyctydine triphosphate (dCTP) and thymidine triphosphate (dTTP) were 107 ±44, 51.1 ±27.2, 53.7 ±31.0 and 108 ±27 pmol/million, respectively, and of adenosine triphosphate (ATP), guanosine triphosphate (GTP), cytidine triphosphate (CTP) and uridine triphosphate (UTP) were 3,570 ±1,840, 1,050 ±550, 606 ±4127 and 1,690 ±3900, respectively (mean ±SD of at least three independent experiments done in duplicate). Values represent the mean ±SE of at least three independent experiments done in duplicate for each treatment condition. Significance from no treatment control on the basis of unpaired two-tailed Student’s t-test assuming equal variance: *<p><0.001, †<p><0.01, ‡<p><0.05.
Effect of nucleoside and nucleotide analogues on nucleotide pools

In order to understand the effect of pharmacologically relevant concentrations of N(t)RTI or their combinations on nucleotide pools, CEM-CCRF cells were treated for 24 h with 10 μM N(t)RTI and endogenous nucleotide pools were quantitated. All treatments that included AZT caused significant increases in dATP, dGTP and TTP up to 1.44-fold more than those observed in untreated cells with the exception of AZT alone and AZT/3TC, where increases in dATP did not reach statistical significance (Table 2). ABC, 3TC, TFV and ddI when incubated alone or ABC/3TC, TFV/ABC, TFV/3TC, TFV/ddI, ddI/3TC, TFV/ABC/3TC and TFV/ddI/3TC when incubated in combination did not show a significant effect on dNTP (data not shown). Furthermore, ABC and 3TC did not show any additional effect on dNTP when combined with AZT over those observed with AZT alone. No N(t)RTI was found to significantly alter rNTP levels (data not shown).

Concentration-dependent effect of AZT on thymidine salvage

To further understand the effect of AZT on thymidine salvage, the concentration-dependent effect of AZT in the presence of 10 μM [13C15N]thymidine on thymidine phosphorylation and nucleotide pools was assessed (Figure 2). The [13C15N]thymidine with stable isotopes incorporated in the base and the sugar allowed for the observation of thymidine kinase and thymidilate kinase-dependent formation of [13C15N]TTP. Treatment of cells with 10 μM [13C15N]thymidine alone did not affect endogenous pyrimidine dNTP levels, but dATP and dGTP levels were 0.7- and 0.5-fold the levels observed in untreated cells, respectively. In contrast to cells treated with 10 μM AZT alone, the presence of [13C15N]thymidine resulted in no effect of 10 μM AZT on dNTP pools. Decreased AZT phosphorylation caused by competition for phosphorylating enzymes by exogenous thymidine and partial abrogation of its activity has been reported previously [29]. In the presence of [13C15N]thymidine, AZT caused a concentration-dependent increase in dCTP when incubated at higher than pharmacological concentrations of 100 and 1,000 μM. Treatment with 100 μM AZT caused increased levels of endogenous TTP, by contrast, treatment with 1,000 μM resulted in significant decreases in the levels of TTP, dATP and dGTP. Salvage of [13C15N]thymidine was only significantly inhibited at the highest concentration of AZT tested (Figure 2B).

Effect of TFV on PNP activity

PNP inhibition is most readily observed in cultured cells by treating with exogenous 2′-deoxyguanosine and determining if dGTP and, to a lesser extent, dATP are increased in the presence of a potential inhibitor [16]. To test the relative ability of TFV to alter dNTP pools through PNP inhibition, CEM-CCRF cells were treated with exogenous 2′-deoxyguanosine and either TFV or PNP405, a potent inhibitor of PNP, for 24 h and dNTP pools were quantified. The addition of exogenous 2′-deoxyguanosine, TFV, or both, did not cause a significant change in dNTP pools (data not shown and Figure 3, respectively). PNP405 alone had no effect on nucleotide pools (data not shown); however, the addition of exogenous 2′-deoxyguanosine caused a large increase in dGTP, a slightly smaller increase in dATP and marked decreases in dCTP and TTP.

Table 2. Effect of 10 μM AZT either alone or in combination on endogenous dNTP pools in CEM-CCRF cells

<table>
<thead>
<tr>
<th>Combination</th>
<th>N(t)RTI†</th>
<th>dATP</th>
<th>dGTP</th>
<th>dCTP</th>
<th>TTP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single</td>
<td>AZT</td>
<td>1.15 ±0.17*</td>
<td>1.38 ±0.18*</td>
<td>1.24 ±0.31</td>
<td>1.44 ±0.22*</td>
</tr>
<tr>
<td>Double</td>
<td>AZT/3TC</td>
<td>1.13 ±0.21*</td>
<td>1.32 ±0.20*</td>
<td>1.14 ±0.25</td>
<td>1.31 ±0.28*</td>
</tr>
<tr>
<td></td>
<td>AZT/ABC</td>
<td>1.15 ±0.10*</td>
<td>1.25 ±0.22*</td>
<td>1.02 ±0.13</td>
<td>1.50 ±0.18*</td>
</tr>
<tr>
<td>Triple</td>
<td>AZT/ABC/3TC</td>
<td>1.23 ±0.11*</td>
<td>1.31 ±0.10*</td>
<td>1.05 ±0.12</td>
<td>1.25 ±0.18*</td>
</tr>
</tbody>
</table>

*Fold change in 2′-deoxynucleoside triphosphates (dNTP) or ribonucleoside triphosphates (rNTP) present in a side-by-side no treatment control (fold change = concentration in treated cells divided by concentration in untreated cells). Levels in untreated cells of 2′-deoxyadenosine triphosphate (dATP), 2′-deoxyguanosine triphosphate (dTTP), 2′-deoxycytidine triphosphate (dTTP) and thymidine triphosphate (dTTP) were 107 ±44, 51.1 ±27.2, 53.7 ±31.0, 108 ±27 pmol/million, respectively (mean ±2σ of at least 20 independent experiments done in duplicate). †Tested nucleoside and nucleotide reverse transcriptase inhibitors (N(t)RTI) or their combinations not containing AZT did not show a statistically significant effect on dNTP pools. No effect of N(t)RTI or their combinations was observed on rNTP pools. Values represent the mean ±2σ of at least three independent experiments done in duplicate. Significance of difference from no treatment control on the basis of unpaired two-tailed Student’s t-test assuming equal variance *P<0.001, †P<0.01, ‡P<0.05. ABC, abacavir; AZT, azidothymidine; 3TC, lamivudine.
Discussion

Consistent with a previous report showing changes in dNTP levels after treatment of CEM-CCRF cells with 25 µM AZT [30], we observed modest increases in TTP, dATP and dGTP following a 24 h incubation with 10 µM AZT. Similarly, it has been observed that inhibition of the major replicative DNA polymerases by an agent like aphidicolin causes an increase in intracellular dNTP pools by uncoupling de novo synthesis from consumption during DNA replication [31]. Furthermore, the extent of dNTP pool increase in cells incubated with nucleotide analogues has been related to intracellular activation and potency against polymerase α, δ and ε [32]. The incorporation of a chain-terminating AZTMP by cellular DNA polymerases has been found to be relatively poor in enzymatic assays [33–36]; however, its toxicity to human bone marrow cells in vitro has been shown to correlate with its incorporation into cellular DNA [7]. One factor favouring stable chain-termination of host DNA is the accumulation of high levels of intracellular AZTMP [12], which has been shown to be a potent inhibitor of 3' to 5' exonuclease proofreading [9]. Taken together, our results coupled with previous observations suggest that the reason for increased TTP, dATP and dGTP upon treatment of CEM-CCRF cells with AZT is due to inhibition of host DNA polymerases.

We have previously shown that TFV monophosphate is a competitive inhibitor of human PNP-dependent ddl phosphoryolysis with an inhibition constant (Ki) of 126 nM and incubation of TFV with CEM-CCRF cells was found to cause a dose-dependent inhibition of ddl degradation [14]. These results illustrate PNP inhibition as the most plausible mechanism for the observation of increased ddl exposure when it is coadministered with TFV. However, based on the requirement for near complete inhibition of PNP to affect dNTP pools and T-cell function [38], TFV therapy is not expected to affect endogenous dNTP pools. Consistent with this hypothesis and unlike a more potent PNP inhibitor, in the current report we show that 10 µM TFV was unable to alter dNTP pools following treatment of cells with exogenous 2′-deoxyguanosine.

In contrast to observations made in this study, another report found that ABC or TDF treatment could increase intracellular TTP, dATP and dGTP in primary CD4+ T-cells stimulated with phytohaemagglutinin and interleukin-2, whereas little or no change in dNTP levels were observed in stimulated CD8+ T-cells [39]. Although, the difference in results from the two studies might be in part due to differences in methodology including the cell types studied, it should be noted that Singer et al. [39] chose to incubate cells with TDF. TDF is the clinically used oral prodruk of TFV that loads cells >1,000-fold more efficiently than TFV [40], but TDF is not observed in the systemic circulation [41]. The resulting higher than pharmacological intracellular concentrations of TFV and its phosphorylated metabolites were likely to have played a role in their observations. In this study, we chose to use CEM-CCRF cells because their rapid division and active nucleotide metabolism make them highly sensitive to perturbations in nucleotide metabolism. The sensitivity of these cells was evident in the marked effects of control antimetabolites.

Various hypotheses have been proposed to explain the poor performance of triple N(t)RTI-only regimens.
The results of this report suggest that pharmacodynamic drug interactions caused by increased competing dNTP pools are unlikely to play a role in the higher rates of virological failures observed for these combinations. On the basis of similar triphosphate analogue formation observed for the combinations of ABC/TFV and ddl/TFV in vitro and in clinical pharmacokinetic studies [42–45], an intracellular drug interaction caused by competition for N(t)RTI activation because of potentially overlapping phosphorylation pathways is also an unlikely explanation for the poor performance of triple N(t)RTI therapies. In contrast, there is support for the intrinsic lack of potency for triple N(t)RTI-only regimens, perhaps caused by suboptimal distribution to certain sites of HIV replication. Supporting this hypothesis, higher rates of treatment failure have been observed in patients treated with the regimens of ABC/ddI/dd4T and ABC/3TC/AZT who had viral loads ≥100,000 copies/ml [20,46]. The continued replication of virus likely allows for viral evolution through parallel resistance pathways held in common by two or more of the N(t)RTI being dosed. For example, ABC/ddI/ TDF therapy was associated with high rates of K65R [22], ddl/3TC/TDF and ABC/3TC/TDF selected for M184V with or without K65R [23,24] and ABC/ ddl/dd4T selected for K65R with or without S68G [47]. Recent clinical studies with 3TC/TDF/AZT and ABC/3TC/TDF/AZT suggest that N(t)RTI-only regimens containing AZT appear to be relatively more efficacious than other triple N(t)RTI-only regimens [48,49]. These results might be explained by the orthogonal resistance profile of AZT, which does not include M184V or K65R.

Although N(t)RTIs mechanistically have the potential to alter nucleotide pools, the results reported here suggest that at pharmacologically relevant concentrations tested, N(t)RTI or their combinations do not have an effect on nucleotide pools with the notable exception of AZT. It was also found that TFV does not have sufficient potency against PNP to perturb dNTP pools. The availability of a convenient, accurate and precise method for measurement of nucleotides will allow for the confirmation of these in vitro findings in cells from patients undergoing HIV therapy.

Acknowledgements

The authors would like to thank William J Watkins (Gilead Sciences, Inc.) for overseeing the synthesis of PNP405 and for thoughtful discussion of the data.

Disclosure statement

The authors are all employed by Gilead Sciences, Inc., the marketer of tenofovir disoproxil fumarate (Viread).

References


Effect of nucleoside and nucleotide analogues on nucleotide pools


