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Tenofovir exhibits low cytotoxicity in various human cell types: comparison with other nucleoside reverse transcriptase inhibitors

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Abstract

Clinical studies with tenofovir disoproxil fumarate, an oral prodrug of the nucleotide analog tenofovir, recently approved for the treatment of HIV, have demonstrated antiviral activity and good tolerability in HIV-infected patients. In order to better understand the cytotoxicity profile of tenofovir relative to the other nucleoside reverse transcriptase inhibitors (NRTIs), the in vitro effects of these agents were evaluated in various human cell types. Tenofovir inhibited the proliferation of liver-derived HepG2 cells and normal skeletal muscle cells with CC_{50} values of 398 and 870 μM, respectively. In comparison, ZDV, ddC, ddI, d4T, and abacavir all showed lower CC_{50} values in these two cell types. Evaluation of hematopoietic toxicity revealed that tenofovir was less cytotoxic towards erythroid progenitor cells ($CC_{50} > 200$ μM) than ZDV, d4T, and ddC ($CC_{50} = 0.06–5$ μM). Despite some degree of donor-to-donor variability, the inhibitory activity of the tested NRTIs against myeloid cell lineage, in the order of decreasing severity, was consistently ddC > ZDV > d4T > tenofovir > 3TC. Finally, tenofovir showed substantially weaker effects on proliferation and viability of renal proximal tubule epithelial cells than cidofovir, a related nucleotide analog with the potential to induce renal tubular dysfunction. In conclusion, tenofovir exhibited weak cytotoxic effects in all cell types tested with less in vitro cytotoxicity than the majority of NRTIs currently used for the treatment of HIV disease. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Tenofovir; Nucleoside reverse transcriptase inhibitors (NRTI); Cytotoxicity; Hematopoiesis

1. Introduction

In spite of the introduction of HIV protease inhibitors as a part of highly active antiretroviral therapy, nucleoside reverse transcriptase inhibitors (NRTIs) are considered a key component of the vast majority of combination regimens for

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treatment of HIV disease (Clumeck and De Wit, 2000; Kaufmann and Cooper, 2000). However, effective control of retroviral replication requires long-term therapy, which may often produce a variety of adverse clinical symptoms. Within the class of NRTIs, zidovudine (ZDV) is known to induce toxicity in liver and skeletal muscle tissues manifested as lactic acidosis, hepatic steatosis, and myopathy (Brinkman et al., 1998). Hepatic steatosis and lactic acidosis can also be associated with didanosine (ddI) and stavudine (d4T)-containing antiretroviral regimens (Bissuel et al., 1994; Miller et al., 2000). Zalcitabine (ddC) has been implicated in the induction of neuropathy in HIV-infected patients (Dubinsky et al., 1989) although more recent studies suggested a somewhat lower incidence of this ddC-associated adverse effect than initially established (Carey, 2000). In addition to adverse effects targeting specific solid organ tissues, NRTI-containing regimens may also produce various hematological disorders. In comparison with untreated control groups, anemia and/or neutropenia have been detected at a substantially higher rate in HIV patients treated with ZDV (Richman et al., 1987), d4T (Browne et al., 1993), or ddC (Yarchoan et al., 1988). These effects correlate with observations from a number of in vitro experiments as well as animal studies demonstrating the inhibitory effects of these NRTIs on the expansion of various hematopoietic progenitor lineages differentiated from bone marrow stem cells (Dainiak et al., 1988; Luster et al., 1991; Du et al., 1992; Dornsife and Averett, 1996).

Tenofovir is an acyclic nucleotide analog with potent in vitro and in vivo antiretroviral activity and as such can be considered a unique type of NRTI (Fig. 1) (Cihlar and Bischofberger, 1998).

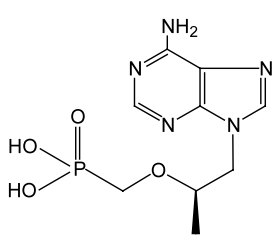

Fig. 1. Structure of tenofovir.

Its lipophilic prodrug, tenofovir disoproxil fumarate, has been recently approved as a novel oral agent for treatment of HIV infection. Several clinical studies with tenofovir disoproxil have demonstrated its potent antiviral efficacy and favorable safety profile in HIV-infected patients (Schooley et al., 2000; Barditch-Crovo et al., 2001). Among related molecules of the same class, cidofovir exhibits clinically proven antiviral activity against human cytomegalovirus (Lalezari et al., 1997). However, unlike tenofovir, anti-cytomegalovirus therapy with cidofovir may be associated with nephrotoxicity, manifested as renal tubular dysfunction, in a proportion of treated patients (Lalezari et al., 1997).

The present study was designed to broaden the understanding of the in vitro toxicity profile of tenofovir by assessing its cytotoxic effects in various cell types of human origin including liver, skeletal muscle, and hematopoietic progenitor cells. In addition, the cytotoxity of tenofovir in renal proximal tubule cells was compared with that of cidofovir in order to better understand the differences in the nephrotoxicity between the two nucleotide analogs. In all cell types tested, tenofovir exhibited only limited cytotoxicity with less inhibitory effects on cellular proliferation than most of the NRTIs currently used for the treatment of HIV infection.

2. Materials and methods

2.1. Drugs

ZDV, d4T, ddC, and ddI were purchased from Sigma (St. Louis, MO), 3TC was supplied by Moravek Biochemicals (Brea, CA), tenofovir was prepared by Gilead Sciences (Foster City, CA) and abacavir was provided by GlaxoWellcome (Research Triangle Park, NC).

2.2. Cytotoxicity in liver and skeletal muscle cells

HepG2 human liver cells (ATCC; Manassas, VA) were maintained in MEM medium supplemented with 10% fetal bovine serum, 1% sodium pyruvate, 0.1 mM non-essential amino acids, and

antibiotics. Primary human skeletal muscle cells (Clonetics, San Diego, CA) were cultured in a medium supplemented by the vendor for a maximum of four passages. To determine drug cytotoxicity, the cells were plated into 96-well plates at a density of 1000 cells/well. Twenty-four hours later, HepG2 and skeletal muscle cells were supplied with fresh medium containing serial dilutions of the tested drugs and incubated for 8 and 6 days, respectively, with replacement of the medium after 3–4 days. Under these conditions, both cell types grew exponentially throughout the duration of the experiments. At the end of the incubation, a modified Neutral Red-based spectrophotometric assay was used to evaluate the cytotoxic effects of the tested NRTIs (Cavanaugh et al., 1990). Briefly, Neutral Red was added to the cells (37.5 μl of 0.3 mg/ml solution in PBS per 150 μl of culture media) and incubated for 90 min at 37 °C. Subsequently, the cells were washed twice with 200 μl PBS per well and the cell-associated dye was extracted with 50 mM Na-citrate/0.05 mM HCl pH 4.2 in 50% ethanol for 30 min at 37 °C. The absorbance was read at 560 nm and the cell growth was expressed as a percentage of the signal relative to the untreated control. The concentration of each drug at which the cell growth was inhibited by 50% (CC_{50}) was estimated from the inhibition plots.

2.3. Hematopoietic toxicity

The assays characterizing the myelotoxicity and erythrotoxicity of NRTIs were performed by using CELISA™ assay (BioWhittaker, Walkersville, MD) according to the following protocol. $CD34^+$ human progenitor cells (Poietic Technologies, Gaithersburg, MD) were purified to at least 95% purity by previously described immunoaffinity techniques (De Wynter et al., 1995) and seeded into 96-well MultiScreen FL filter plates (Millipore Corp., Bedford, MA) at a density of 2000 cells/well in X-VIVO 15 medium (BioWhittaker) containing specific differentiating cytokines. The myeloid lineage was differentiated in the presence of 15% fetal bovine serum, 1 ng/ml G-CSF, 1 ng/ml GM-CSF and 25 ng/ml SCF (all from R&D Systems, Minneapolis, MN). The erythroid lineage was differentiated in growth media containing 10 μg/ml insulin, 200 μg/ml holotransferrin (Intergen, Purchase, NY), 25 ng/ml SCF, and 3 units/ml erythropoietin (R & D Systems, Minneapolis, MN). The test compounds were then added to the cells at five-fold serial dilutions and the incubation was carried out for 10 days. The expansion of the myeloid and erythroid lineages was quantified by determination of lineage-specific cell surface markers CD11b and glycophorin A, respectively. Anti-CD11b and anti-glycophorin A antibodies were produced by specific hybridomas (ATCC, Manassas, VA) and labeled with europium by EG&G Wallac (Akron, OH). Filter plates containing differentiated hematopoietic progenitor cells were placed on a Millipore vacuum manifold adjusted to a vacuum pressure of 5 in. Hg. The cell culture supernatants were removed by vacuum filtration and the anti-CD11b or anti-glycophorin A antibodies were added at 100 μl/well at concentrations of 2 or 4 μg/ml, respectively. After 60 min at room temperature, the wells were vacuum filtered and rinsed three-times with Wash Buffer (PerkinElmer Life Sciences). Enhancement Solution (PerkinElmer Life Sciences) was added (0.1 ml/well) and, after 5 min, europium fluorescence (excitation at 340 nm and emission at 615 nm) was measured over a 400 μs time period after an initial delay of 400 μs with a Wallac Victor spectrofluorimeter. Myelotoxicity and erythrotoxicity was expressed as the percentage inhibition relative to the untreated control lineages with CC_{50} values estimated from the inhibition plots.

2.4. Evaluation of cytotoxicity in renal proximal tubule cells

Primary human renal proximal tubule epithelial cells (RPTECs) were provided by Dr Kenneth McMartin (Louisiana State University, Shreveport, LA) and maintained on plastic dishes coated with Vitrogen-100 (Collagen Biomaterials; Palo Alto, CA). RPTECs were grown for a maximum of three passages in DMEM/F-12 (1:1) medium supplemented with 5 ng/ml selenium, 5 μg/ml insulin, 5 μg/ml transferrin, 40 ng/ml hydrocortisone, 10 ng/ml epidermal growth factor (Collabo-

rative Research, Bedford, MA), and 4 pg/ml triiodothyronine (Morshed and McMartin, 1995). Cytotoxicity experiments with RPTECs were carried out in 12-well plates with cells seeded at a density of 20 000 cells/well 24 h prior to the addition of the tested drugs. Following a 4-day incubation with exponentially growing cells, the culture was trypsinized and cells counted. The CC_{50} values were determined from a percentage of cells relative to untreated control. The assay for evaluation of the viability of quiescent RPTECs was performed in 96-well format. The cells were seeded at a density of 50 000 cells/well and incubated for 3–4 days until full confluence was reached. At that time, the drugs were added at a concentration of 500 μM (day 0) and the cells were incubated in the presence of tested drugs for 5, 10, 15, 20, and 25 days with a regular media change every 5 days. At the end of each incubation, cell viability was determined by using the MTT-based assay (Carmichael et al., 1987) and the half-life ($T_{1/2}$) of the cultured cells in the presence and absence of each drug was estimated as the time point at which the cell viability was reduced by 50% relative to day 0.

3. Results

3.1. Cytotoxicity of tenofovir in human liver and skeletal muscle cells

Adverse symptoms associated with use of NRTIs such as hepatic steatosis, muscle myopathy, or lactic acidosis are primarily a consequence of drug-associated effects on liver and skeletal muscle tissues. Therefore, the in vitro cytotoxicity of tenofovir was determined in HepG2 human liver cells and human skeletal muscle cells and directly compared with that of other NRTIs. Tenofovir after an 8-day incubation with the proliferating HepG2 cells showed only a weak inhibition of the cell growth as indicated by its CC_{50} of 399 μM (Table 1). Under the same conditions, 3TC was less cytotoxic than tenofovir. Abacavir, d4T, ddI, ZDV, and ddC caused more pronounced inhibition of HepG2 cell growth than tenofovir with ddC being the most cytotoxic drug amongst all the NRTIs tested ($CC_{50} = 7.7$ μM). In contrast with all other cell types used in this study, HepG2 represent a transformed non-primary cell line. Thus, the in vitro data generated with these cells should be interpreted more cautiously with respect to predicting in vivo behavior of the tested drugs.

Table 1
In vitro cytotoxicity of tenofovir and other NRTIs in human HepG2 liver cells and skeletal muscle cells

Drug	In vitro cytotoxicity-CC_{50} (μM)[a]	
	HepG2 cells	Skeletal muscle cells
Tenofovir	399 ± 31	870 ± 275
Abacavir	320 ± 35	97 ± 13
ZDV	87 ± 11	497 ± 19
ddC	7.7 ± 0.2	90 ± 38
ddI	360 ± 70	846 ± 253
d4T	290 ± 22	66 ± 38
3TC	1020 ± 66	1230 ± 331

[a] Exponentially growing HepG2 cells and skeletal muscle cells were incubated in the presence of the tested drugs for 8 and 6 days, respectively, and CC_{50} values were determined using Neutral Red-based spectrophotometric assay. The data are means ± standard deviations from two independent experiments performed in triplicate.

Similarly to HepG2 liver cells, low in vitro cytotoxicity of tenofovir was also observed in skeletal muscle cells. In a 6-day assay, tenofovir showed weak inhibition of cell growth with CC_{50} of 870 μM, an effect comparable to that of 3TC and ddI (Table 1). In contrast, ZDV was somewhat more cytotoxic than tenofovir. Abacavir, ddC, and d4T with $CC_{50} < 100$ μM were substantially more cytotoxic than tenofovir in skeletal muscle cells.

3.2. Hematopoietic toxicity of tenofovir and other NRTIs

Because of its continual cell proliferating activity, the hematopoietic system is often adversely affected by cytotoxicity associated with various therapeutic agents. The hematopoietic toxicity of tenofovir and four other NRTIs (ZDV, d4T, ddC, and 3TC) was evaluated in human $CD34^+$ bone marrow progenitor stem cells exposed to specific

cytokines, which programmed their differentiation and expansion into the erythroid and myeloid lineages. The expansion of the two lineages in the presence of the tested drugs was determined with progenitor cells from two independent donors by immunofluorescence detection of lineage-specific cell surface markers. Irrespective of the donor, tenofovir, at concentrations as high as 200 μM, showed no significant effects on the expansion of the erythroid lineage from the progenitor stem cells as determined by the level of expression of glycophorin A (Table 2). Likewise, tenofovir showed only limited effects on the expansion of the myeloid lineage based on the expression of CD11b with the inhibition being more pronounced in progenitor cells from donor 2 (CC_{50} of 85 μM). Expansion of the myeloid lineage from donor 1 was less affected by tenofovir ($CC_{50} > 200$ μM). Likewise, 3TC exhibited only a weak cytotoxicity against both the erythroid and myeloid lineages with a moderate degree of inhibition observed at a concentration of 200 μM. In contrast, ZDV and d4T produced a substantial suppression of the erythroid lineage expansion with CC_{50} values 0.62–0.85 μM and 3.3–5 μM, respectively. In addition, ZDV induced 50% suppression of the myeloid lineage from donors 1 and 2 at concentrations 49 and 3.6 μM, respectively. Similarly, d4T was only marginally myelotoxic in cells from donor 1, but it produced a substantial myelosuppression in cells from donor 2 ($CC_{50} = 10.5$ μM). In comparison with the other tested NRTIs, ddC caused by far the most severe suppression of both the erythroid and myeloid lineages with CC_{50} values ranging from < 0.06 to 0.38 μM.

Overall, the drug susceptibility of the erythroid lineage was similar in cells from both donors. However, there was consistently more pronounced myelotoxicity detected with each tested drug in the progenitor cells from donor 2 emphasizing potential patient-to-patient variations in the sensitivity to drug-associated hematopoietic disorders (Table 2).

Table 2
In vitro hematopoietic toxicity of tenofovir in comparison with other NRTIs[a]

Drug	Hematopoietic toxicity-CC_{50} (μM)[b]			
	Myeloid lineage		Erythroid lineage	
	Donor 1	Donor 2	Donor 1	Donor 2
Tenofovir	>200	85	>200	>200
ZDV	49	3.6	0.85	0.62
d4T	200	10.5	5.0	3.3
ddC	0.38	0.24	0.14	<0.06
3TC	>200	140	>200	170

[a] Production of the myeloid and erythroid lineage was determined using a lineage-specific marker immunoassay following a 10-day incubation of stimulated human bone marrow progenitor cells in the presence of the tested drugs.

[b] Concentration of each drug inhibiting production of the myeloid or erythroid lineage from progenitor stem cells by 50%. The results are from a representative experiment performed in triplicate. Standard error for each data point was <20% of the average.

3.3. Cytotoxicity of tenofovir in renal proximal tubule epithelial cells

The cytotoxic effects of tenofovir were also determined in normal human renal proximal tubule epithelial cells (RPTECs) and compared with those of cidofovir in order to better understand the differences in nephrotoxicity observed in vivo among the two structurally related acyclic nucleotide analogs.

As in the other cell types, the in vitro growth of RPTECs was only marginally affected by tenofovir as indicated by its $CC_{50} > 2000$ μM (Fig. 2a). In comparison, cidofovir showed more pronounced cytotoxic effects in RPTECs with a CC_{50} value of 260 ± 42 μM ($n = 2$). Likewise, the results of experiments evaluating the viability of quiescent RPTECs in the presence of the two nucleotide analogs indicated that tenofovir at a concentration of 500 μM had no substantial effect on cell viability during a prolonged 25-day exposure as demonstrated by no substantial differences between the viability of the cells treated with tenofovir and that of the untreated control cells (Fig. 2b). In contrast, the half-life of quiescent RPTECs in the presence of 500 μM cidofovir was 9.7 ± 2.2 days ($n = 2$).

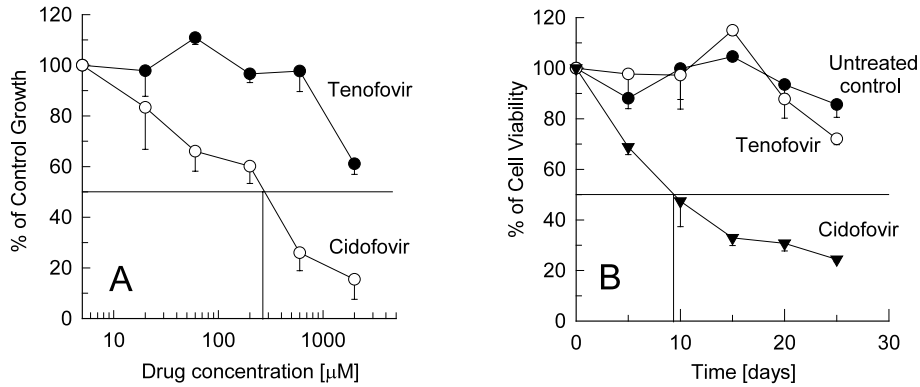

Fig. 2. Effects of tenofovir and cidofovir on the human renal proximal tubule epithelial cells. (A) Inhibition of cell proliferation. Cell growth was determined by the counting of cells after a 4-day incubation in the presence of the tested drugs and expressed as percentage of the untreated control. (B) Effects on the viability of quiescent cells. Contact-inhibited renal cells were incubated in the presence of each drug for 5–25 days; cell viability was determined every 5 days by using the MTT-based spectrophotometric assay and expressed as percentage of signal at the beginning of the treatment (day 0). The data represent means ± standard deviations from a representative experiment performed in duplicate (A) or triplicate (B).

4. Discussion

Several clinical studies have demonstrated the antiviral efficacy and tolerability of tenofovir (Schooley et al., 2000; Barditch-Crovo et al., 2001). In order to characterize the relative cytotoxic potential of tenofovir within the class of NRTIs, a study was conducted comparing the in vitro effects of tenofovir on different cell types with the other clinically used NRTIs. In human liver and skeletal muscle cells, tenofovir showed only weak inhibition of cell proliferation with CC_{50} values exceeding 300 μM. In comparison with the other six clinically relevant NRTIs, tenofovir was the second least cytotoxic agent after 3TC. Prior in vitro and in vivo studies have indicated that some NRTIs affect mitochondrial functions, which presumably plays a primary role in the etiology of NRTI-associated adverse effects occurring in the liver and skeletal muscle tissues (Brinkman et al., 1998; Kakuda, 2000). Recently, we have conducted a study in both skeletal muscle cells and HepG2 liver cells demonstrating the minimal mitochondrial toxicity of tenofovir as assessed by its effects on the synthesis of mitochondrial DNA and lactic acid production (Birkus et al., 2001). In contrast, ddC, ddI, d4T, and AZT have been shown to affect either mitochondrial DNA levels or the production of lactic acid (Brinkman et al., 1998; Kakuda, 2000). Thus, the cytotoxicity of these NRTIs may be at least in part due to their effects on mitochondrial functions. Consistently, 3TC, which showed only minimal cytotoxicity, does not adversely affect mitochondrial functions in vitro (Martin et al., 1994; Pan-Zhou et al., 2000).

Hematological disorders such as anemia and neutropenia were amongst the first recognized adverse effects associated initially with ZDV therapy and then later also with d4T and ddC therapy (Richman et al., 1987; Yarchoan et al., 1988; Browne et al., 1993). Analysis of the in vitro expansion of specific hematopoietic progenitors from human bone marrow stem cells revealed no significant inhibition of the erythroid lineage in the presence of tenofovir at concentrations as high as 200 μM. Although the myeloid progenitors appeared to be more susceptible to tenofovir, the effects were comparable to that of 3TC with no substantial inhibition of the myeloid lineage expansion at 40 μM tenofovir. Drug exposure ($AUC_{0-\infty}$) following oral administration of the recommended therapeutic doses is similar for tenofovir, ZDV, d4T, and 3TC while the therapeutically relevant dose of ddC produces AUC lower than that observed with the other four

NRTIs (Table 3). Importantly however, the peak plasma concentrations (C_{max}) for ZDV, d4T, and ddC are in the range of those showing substantial hematopoietic toxicity. In comparison, the therapeutic dose of the tenofovir prodrug in HIV patients delivers free tenofovir into the systemic circulation with C_{max} of 240–374 ng/ml (0.8–1.3 μM) (Barditch-Crovo et al., 2001). Thus, the effects of tenofovir on the erythroid and myeloid progenitor cells were detected at concentrations significantly exceeding the therapeutically relevant plasma level. Taken in total, these conclusions are consistent with clinical data demonstrating the lack of hematological disorders in patients treated with tenofovir oral prodrug for >48 weeks (Schooley et al., 2000) and may explain, at least in part, the hematological adverse effects observed during treatment with some of the other NRTIs (Richman et al., 1987; Yarchoan et al., 1988; Browne et al., 1993).

The limited effects of tenofovir and 3TC on the two progenitor lineages were in contrast with those of the other tested NRTIs. For example, ddC inhibited the expansion of both the erythroid and myeloid lineages at sub-micromolar concentrations. Likewise, ZDV and d4T exhibited substantial cytotoxicity towards the erythroid progenitor cells at sub-micromolar and low micromolar concentrations, respectively. The inhibition potency of ZDV, d4T, ddC, and 3TC against the myeloid and erythroid lineages, determined in this study by using the quantitative detection of the lineage-specific cell surface antigens, correlates with prior data from studies based on the standard colony-forming assays (Dornsife and Averett, 1996).

Similar to its effects on the other cell types, the effects of ddC on the expansion of hematopoietic progenitors may be related to its severe mitochondrial toxicity due to the depletion of mitochondrial DNA (Medina et al., 1994; Rossi et al., 1999; Pan-Zhou et al., 2000). In contrast, the substantial inhibition of the erythroid progenitors by ZDV may be associated with the potent effects of ZDV on the cellular biosynthesis of branched N-linked oligosaccharides present in membrane glycoproteins and glycosphingolipids (Yan et al., 1995; Steet et al., 1999, 2000). Glycosylation of proteins and lipids is believed to be an important component of the differentiation program of various eukaryotic cell types. Importantly, the differentiation of hematopoietic stem cells is strongly associated with the specific changes in the glycosylation patterns of proteoglycans and glycolipids (Gahmberg et al., 1988; Drzeniek et al., 1999).

Previous clinical studies with cidofovir identified nephrotoxicity as the main clinical adverse effect associated with this antiviral nucleotide analog (Lalezari et al., 1997). In contrast, trials with tenofovir have demonstrated its favorable safety profile with no significant signs of renal dysfunction in patients treated with prolonged tenofovir therapy (Schooley et al., 2000; Barditch-Crovo et al., 2001). hOAT1, a membrane transport protein localized specifically in the basolateral membrane of the renal proximal tubule epithelium, has been implicated in the etiology of cidofovir-associated nephrotoxicity by mediating efficient uptake of the drug into the renal proximal epithelium (Ho et al., 2000). Transport kinetic experiments revealed a similar

Table 3
Pharmacokinetics of NRTIs in HIV-1 infected patients following oral administration

NRTI	Dose administered (recommended dosing)	C_{max} μg/ml (μM)	$AUC_{0-\infty}$ μg h/ml (μM h)	References
Tenofovir[a]	300 mg (300 mg qd)	0.24–0.37 (0.8–1.3)	2.09–3.18 (7.3–11.1)	Barditch-Crovo et al., 2001
ZDV	300 mg (300 mg bid)	1.64 (6.1)	2.01 (7.5)	Wang et al., 1999
d4T	0.67 mg/kg (40 mg bid)	1.19 (5.3)	1.73 (7.7)	Dudley et al., 1992
ddC	1.5 mg (0.75 mg tid)	0.016–0.025 (0.08–0.12)	0.062–0.072 (0.29–0.34)	Nazareno et al., 1995
3TC	150 mg (150 mg bid)	1.50 (6.6)	5.31 (23.2)	Wang et al., 1999

[a] Administered as an oral prodrug tenofovir DF.

efficiency of hOAT1-mediated transport for cidofovir and tenofovir suggesting that there may not be any significant differences in renal accumulation of the two drugs that could explain their different nephrotoxic potentials (Cihlar et al., 2001). However, direct comparison of the effects of cidofovir and tenofovir on the growth and viability of renal proximal tubule cells revealed significant differences between the cytotoxicity of the two-nucleotide analogs. These differences correlated with the relative potential of these two agents to cause in vivo renal tubular dysfunction and further indicate that the degree of interference with essential cellular functions rather than the level of renal accumulation is presumably responsible for the differences in the nephrotoxic potential between cidofovir and tenofovir.

In conclusion, this study, which was focused on the in vitro evaluation of tenofovir in several human cell types, provided comprehensive evidence of the low in vitro cytotoxicity of tenofovir. In contrast, the majority of the other NRTIs currently used as part of combination antiretroviral therapy, exhibited more pronounced cytotoxic effects in the human cells tested. These results are consistent with the favorable tolerability profile of tenofovir in HIV-infected patients.

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