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Mini-review

Pharmacology of current and promising nucleosides for the treatment of human immunodeficiency viruses

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Dedicated to Prof. Erik De Clercq on the occasion of reaching the status of Emeritus-Professor at the Katholieke Universiteit Leuven in September 2006.

Abstract

Nucleoside antiretroviral agents are chiral small molecules that have distinct advantages compared to other classes including long intracellular half-lives, low protein binding, sustained antiviral response when a dose is missed, and ease of chemical manufacture. They mimic natural nucleosides and target a unique but complex viral polymerase that is essential for viral replication. They remain the cornerstone of highly active antiretroviral therapy (HAART) and are usually combined with non-nucleoside reverese transcriptase and protease inhibitors to provide powerful antiviral responses to prevent or delay the emergence of drug-resistant human immunodeficiency virus (HIV). The pharmacological and virological properties of a selected group of nucleoside analogs are described. Some of the newer nucleoside analogs have a high genetic barrier to resistance development. The lessons learned are that each nucleoside analog should be treated as a unique molecule since any structural modification, including a change in the enantiomeric form, can affect metabolism, pharmacokinetics, efficacy, toxicity and resistance profile.

Keywords: Antiviral agents; Pharmacology; Nucleoside analogs; HIV

Contents

Nucleoside analogs as a class have a well-established regulatory history, with 11 currently approved by the US Food and Drug Administration (US FDA) for the treatment of human immunodeficiency virus (HIV), hepatitis B virus (HBV) or hepatitis C virus (HCV). The challenge in developing antiviral therapies is to inhibit viral replication without injuring the host cell. In HIV, a major key target for drug development is HIV reverse transcriptase (HIV-RT), a unique viral polymerase. This enzyme is active early in the viral replication cycle and converts the virus' genetic information, which is stored as RNA into DNA by reverse transcription, a process necessary for contin-

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ued viral replication. Nucleoside reverse transcriptase inhibitors (NRTI) mimic natural nucleosides. In the triphosphate form, each NRTI competes with one of the four naturally occurring 2′-deoxynucleoside 5′-triphosphates (dNTP), namely, dCTP, TTP, dATP or dGTP, for binding and DNA chain elongation near the active site of HIV-1 RT.

By 2006, eight HIV NRTI were approved by the US FDA including: Zidovudine® (ZDV, AZT), Videx® (ddI, didanosine), Hivid® (ddC, zalcitabine), Zerit® (d4T, stavudine), Ziagen® (abacavir sulfate, ABC), Epivir® (3TC, lamivudine), Emtriva® (FTC, emtricitabine) and Viread® (tenofovir disoproxil fumarate, TDF). These NRTI are the backbone of modern highly active antiretroviral therapies (HAART) (Schinazi, 1991; Yeni et al., 2004). Current first line HAART regimens combine two NRTI together with either a protease inhibitor (PI) or non-nucleoside reverse transcriptase inhibitor (NNRTI). These drug cocktails have markedly decreased mortality and morbidity from HIV-1 infections in the developed world (Carpenter et al., 2000; Erickson-Viitanen et al., 2003). Existing therapies cannot eradicate HIV infection because of the compartmentalization of the virus and its latent properties (Siliciano and Siliciano, 2004; Siliciano, 2005). In fact new data indicate that drug holidays in subjects that have undetected HIV in their serum are generally contraindicated and that a backbone of NRTI is essential to suppress viral recrudescence (Deeks et al., 2005). Therefore, chronic therapy remains the standard of care for the foreseeable future. Many regimens eventually fail, due primarily to lack of adherence to strict regimens, delayed toxicities and/or the emergence of drug-resistant HIV strains (Pereira and Paridaen, 2004). This review discusses ZDV, 3TC, FTC, TDF and ABC, five of the most commonly used potent antiretroviral drugs approved by the US FDA (Fig. 1). We will also discuss newer potent NRTI undergoing clinical evaluation, including Racivir [RCV, (±)-β-2′,3′-dideoxy-5-fluoro-3′-thiacytidine], Dexelvucitabine (β-D-2′,3′-didehydro-2′,3′-dideoxy-5-fluorocytidine, reverset, RVT, D-D4FC, DFC), Amdoxovir [AMDX, (−)-β-D-2,6-diaminopurine dioxolane, DAPD], AVX754 [SPD-754, (−)dOTC, (−)-2′-deoxy-3′-oxa-4′-thiocytidine], and two NRTI in preclinical development, 9-(β-D-1,3-dioxolan-4-yl)-2-aminopurine (APD) and (−)-(2*R*,4*R*)-1-(2-hydroxymethyl-1,3-dioxolan-4-yl)thymine (DOT) (Fig. 1).

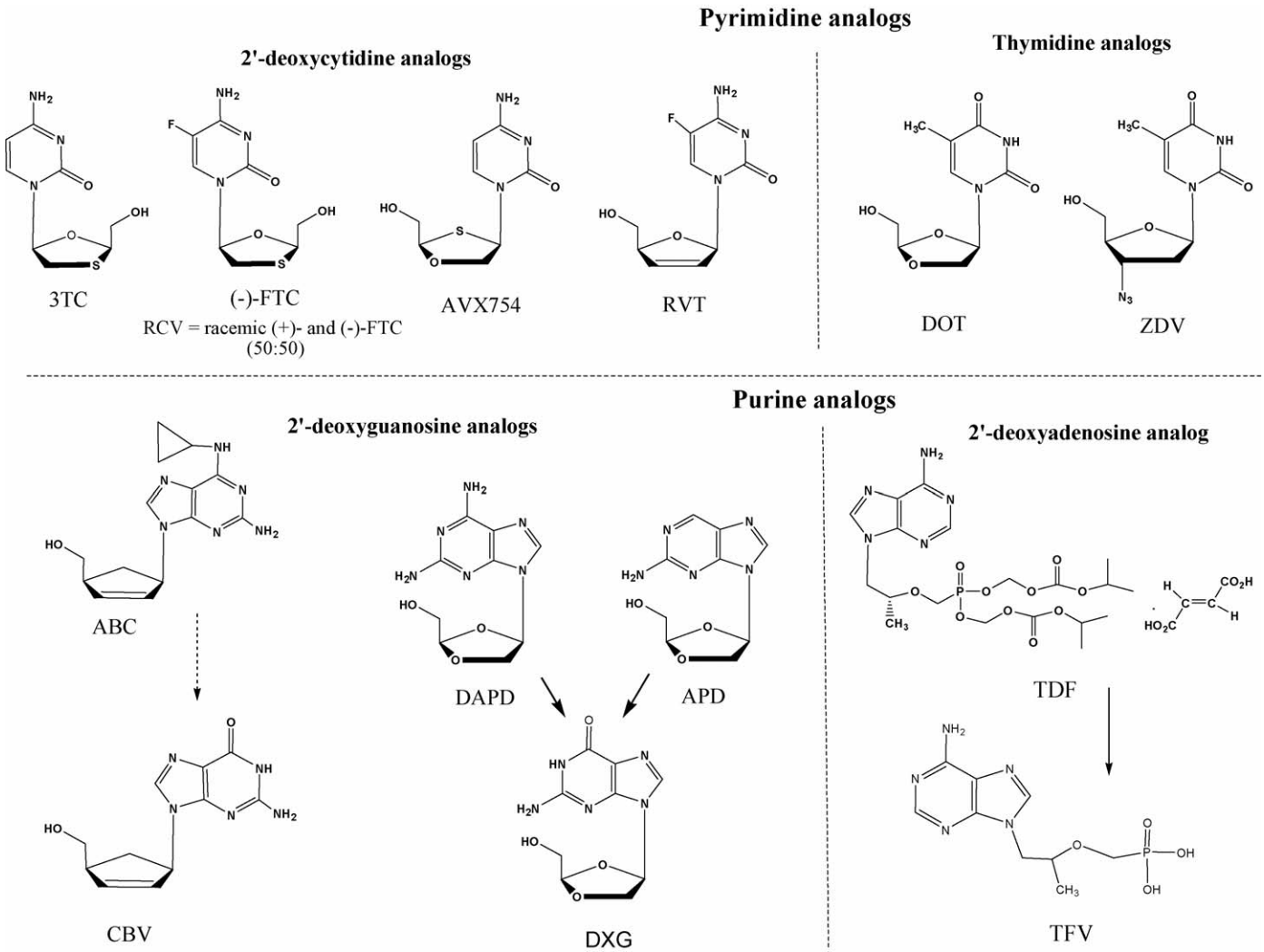

Fig. 1. Chemical structures of nucleoside approved by US FDA and under development.

1. NRTI efficacy

NRTI require phosphorylation by intracellular enzymes to their active fraudulent NRTI triphosphate (NRTI-TP) analog form, which compete with the natural dNTP for incorporation by HIV-1 RT into the elongating proviral DNA. Since all current NRTI lack a 3′-hydroxyl group, incorporation results in chain termination (Lipsky, 1996). The fact that a specific NRTI-TP competes with its natural nucleotide each time it appears in the active site near the reverse-transcribing viral RNA chain, produces multiple opportunities for NRTI blockade during a single round of reverse transcription. The long intracellular half-life of many NRTI-TP can result in a significant post-antibiotic effect, which may sometimes be exploited for designing more convenient dose regimens that are more forgiving when it comes to missed doses compared to PI and NNRTI (Sommadossi, 1982). Therefore, unlike NNRTI and PI, in which the antiviral pharmacodynamics is directly predicted by the between dose trough plasma concentrations (C_{min}) (Acosta et al., 1999; Fletcher, 1999; Fletcher et al., 2000; Lorenzi et al., 1997; Stein et al., 1996), the efficacy of NRTI may be more related to maximal plasma concentrations (C_{max}), which would drive cellular accumulation and phosphorylation of the NRTI to the long-lived triphosphate, unless phosphorylation is rate limiting (e.g., see ZDV below).

Factors that influence NRTI efficacy and dose frequency include: the drug concentration versus time profile in plasma (pharmacokinetics), the efficiency of cellular uptake of the NRTI levels of phosphorylation to NRTI-TP, and its inhibition constant (K_i) with the HIV-1 RT. For example, (−)-FTC has an 8–10 h half-life ($t_{1/2}$) in the plasma (Table 1). Therefore, based on an expected 24 h trough concentration in plasma, a once a day dosing regimen may seem inadequate (∼4 × plasma $t_{1/2}$ value = 12% of the C_{max} value). However, cellular levels of FTC-TP reach levels of ∼4 pmol/10^6 cells (approximately 20 μM, assuming a volume of 0.1 μl/10^6 cells), which is much greater than the K_i value (1.4 μM) versus HIV-1 RT (Feng et al., 1999). The $t_{1/2}$ of the active FTC-TP is 29–56 h (Wang et al., 2004). Therefore, (−)-FTC can be dosed once daily. Likewise, 3TC accumulates 17.5 ± 6.4 pmol of 3TC-TP per 10^6 primary human peripheral blood mononuclear (PBM) cells (approximately 87.5 μM) and the K_i value is 5 μM (Feng et al., 1999), and has an intracellular $t_{1/2}$ of 15–16 h. Therefore, 3TC may be administered once daily (300 mg) or twice daily (150 mg) (Solas et al., 1998). The active metabolite of TDF, TFV-diphosphate (TFV-DP), has an intracellular $t_{1/2}$ of 60 to greater than 175 h, which strongly supports once-daily dosing (Back et al., 2005; Hawkins et al., 2005; Moore et al., 1999b; Yuen et al., 2004). Plasma pharmacokinetics, recommended oral doses, maximal cellular concentrations, half-lives of the various NTP, K_i values, and expected maximal viral load reductions, when used in monotherapy, and common resistance mutations of the various NRTI in humans are summarized in Table 1.

In vitro studies suggest that it is preferable to combine NRTI that employ different initial kinases, to avoid competitive inhibition of their respective phosphorylation enzymes (Bethell et al., 2003, 2004a,b, 2005; Havlir et al., 2000). For example, 3TC and FTC interfere with the phosphorylation of the 2′-deoxycytidine analog AVX754, resulting in a significant reduction in its active NTP (AVX754-TP), in primary human PBM cells *in vitro* and in PBM cells isolated from treated subjects (Bethell et al., 2004a,b, 2005). Similarly, the combination of the thymidine analogs ZDV and D4T, is contraindicated in the clinic, since both use cytosolic thymidine kinase (TK1) for their initial phosphorylation (Havlir et al., 2000). However, this dogma may not necessarily hold true for all nucleosides. Co-incubation of RVT with 3TC at clinically relevant concentrations, does not decrease the accumulation of 3TC-TP in T-cell lymphoma (CEM) or primary human PBM cells. This may be related to the similarities in K_m values towards 2′-deoxycytidine kinase (dCK), since neither compound was able to displace the other efficiently. Further, intracellular levels of RVT-TP were only decreased at 3TC concentrations greater than those observed in the clinic in CEM cells (Hernandez-Santiago et al., 2004). Nevertheless, caution is warranted when using nucleoside analogs that share the same initial kinase. Possible strategies for overcoming competitive phosphorylation may include staggering the times of administration, thereby decreasing the total substrate load (sum of C_{max} values in plasma) on the enzymes, or increasing the dose of the agent less able to compete (RVT in this case) (Hernandez-Santiago et al., 2004). However, such strategies remain to be tested in carefully designed clinical trials since the intracellular $t_{1/2}$ of RVT and 3TC is high.

2. NRTI mitochondrial toxicity

HIV infection may be associated with reduction in mitochondrial DNA content and changes in mitochondrial morphology and function, which in some cases leads to clinical events such as myopathy or peripheral neuropathy (Moyle, 2005). Therefore, the toxicity of NRTI *in vivo* and *in vitro* are at least partially related to inhibition of mitochondrial DNA polymerase gamma, the sole enzyme responsible for the base excision repair of oxidative damage to mitochondrial DNA (Fromenty and Pessayre, 1997; Johnson et al., 2001; Kakuda, 2000; Lewis et al., 2006; Martin et al., 2003; Moyle, 2000; Zapor et al., 2004). This toxicity may be associated with a decrease in mitochondrial DNA and disrupted oxidative phosphorylation, evidenced by the toxic accumulation of non-esterified fatty acids, dicarboxylic acids and free radical damage (Kakuda, 2000). Published constants of inhibition of mitochondrial DNA polymerase gamma ($K_{i\text{-DNA pol }\gamma}$) derived using non-steady state kinetic measurement by various NRTI-TP are summarized in Table 1 (Feng et al., 2004a; Furman et al., 2001; Johnson et al., 2001; Murakami et al., 2004). Non-steady state values of $K_{i\text{-DNA pol }\gamma}$ were quoted, since values determined using steady state methods may be unreliable due to the processive nature of DNA polymerases (Lee et al., 2003). Another more direct *in vitro* predictor of toxicity due to inhibition of $K_{i\text{-DNA pol }\gamma}$ by NRTI is the *in vitro* toxicity index (TI) (Johnson et al., 2001; Lee et al., 2003). TI is defined as the relative increase in the time required to replicating the mitochondrial genome based on the rates of incorporation and removal of

Table 1
Pharmacological properties of selected nucleoside reverse transcriptase inhibitors (NRTI)

	NRTI								
	FDA approved					Clinical trial			
	ZDV[a]	3TC[b]	(−)-FTC[c]	TDF[d]	ABC/CBV[e]	RCV[f]	RVT[g]	DAPD/DXG[h]	AVX-754[i]
Sponsor	GSK	GSK/Shire	Gilead sciences	Gilead sciences	GSK	Pharmasset Inc.	Pharmasset Inc.	RFS Pharma LLC	Avexa Ltd.
Competing natural nucleosides	dThd	dCyd	dCyd	dAdo	dGuo	dCyd	dCyd	dGuo	dCyd
Mean single dose AUC (μg/ml × h)	2.01	16.6	11.8 ± 2.9	2.29–3.02	5.31–5.48	10.7 ± 1.9	11.73 ± 2.08	N/A[j]	N/A
C_{max} (μg/ml)	1.64	3.5	2.2 ± 0.6	3.26	0.64–4.38	2.6 ± 0.4	1.75 ± 0.36	N/A	N/A
$t_{1/2}$ in plasma (h)	0.5–3	5–7	8–10	12–17	1.54 ± 0.63	6.67 ± 1.48	5.2	4–7	N/A
EC_{50} vs. HIV-1 in stimulated PBM cells (μM)	0.01–0.09	0.003–0.15	0.001–0.69	0.01–0.02	0.91–0.98	0.007–0.087	0.6 ± 0.3	DAPD 4; DXG 0.11–0.25	N/A
Intracellular $t_{1/2}$ of NTP (in HIV infected humans, h)	3–4	15–16	29–56	60 to >175	12–21	N/A	13–17 (in vitro)	9.4	6–7
K_i of NTP vs. wild type HIV-1-RT enzyme (μM)	0.035	5.0 ± 0.8	1.4	0.21 ± 0.04	0.11 ± 0.03	N/A	0.1	0.05	N/A
$K_{i\text{-DNA pol }\gamma}$ (μM)	187 ± 54	9.2 ± 0.9	62.9 ± 8.4	40.3 ± 5.7	13.0 ± 1.5	N/A	0.82 ± 0.2	4.3 ± 0.4	N/A
Toxicity index[k] (in vitro)	1.05	1.2	N/A	2.1	1.0	N/A	662	N/A	N/A
NTP-levels in PBM cells (pmol/10^6 cells) measured infected humans	0.07–0.024	17.5 ± 6.4	1.9–4	0.085–0.11	0.09–0.20	N/A	N/A	0.7	1.65–6.10
Recommended oral dose for HIV	300 mg bid	300 mg qd and 150 mg bid	200 mg qd	300 mg qd	600 mg qd	600 mg qd	200 mg qd	500 mg bid	200–800 mg bid 800–1200 mg qd
Maximal $\log_{10}$—copies/ml reduction in viral load (monotherapy)	0.85 (14 days)	2.25 (14 days)	1.9 (14 days)	1.5 (14 days)	1.1–1.8 (4 weeks)	2.0–2.4 (day 14)	1.77 (10 days)	1.3–1.5 (14 days)	1.65 (10 days)
Signature resistance mutations selected in vitro	TAMs	M184V	M184V	K65R	M184V, L74V, K65R, Y115F	M184V	K65R	K65R or L74V	K65R, V75I, M184V
Activity vs. HBV in culture	No	Yes	Yes	Yes	No	Yes	No	Yes	N/A
Phase of development	Approved 1986, generic 2005	Approved 1995	Approved 2003	Approved 2001	Approved 1998	2b	2b	2b	2b

Abbreviations: Zidovudine® (ZDV, AZT), Epivir® (3TC, lamivudine), Emtriva® (FTC, emtricitabine), Viread® (tenofovir disoproxil fumarate, TDF), Ziagen® (abacavir sulfate, ABC), Carbovir® (CBV), Racivir [RCV, (±)-β-2′,3′-dideoxy-5-fluoro-3′-thiacytidine], Dexelvucitabine® (β-D-2′,3′-didehydro-2′,3′-dideoxy-5-fluorocytidine, reverset, RVT, D-D4FC, DFC), Amdoxovir® [AMDX, (−)-β-D-2,6-diaminopurine dioxolane, DAPD], 9-(beta-D-1,3-Dioxolan-4-yl)guanine (DXG), AVX754 [SPD-754, (−)dOTC, (−)-2′-deoxy-3′-oxa-4′-thiocytidine].

[a] [Combivir package insert. GlaxoSmithkline]; (Anderson et al., 2004; Back et al., 2005; Barreiro et al., 2004; Cannard et al., 1998; Johnson et al., 2001; Murakami et al., 2004; Piliero, 2004; Reardon and Miller, 1990; Sharma et al., 2004; Wang et al., 1999).

[b] [Combivir package insert. GlaxoSmithkline]; (Anderson et al., 2004; Back et al., 2005; Barreiro et al., 2004; Hawkins et al., 2005; Herzmann et al., 2005; Johnson et al., 1999, 2001; Moore et al., 1999b; Murakami et al., 2004; Piliero, 2004; Sharma et al., 2004; Solas et al., 1998; Wang et al., 1999; Yuen et al., 2004).

[c] [Emtriva package insert. Gilead Sciences]; (Anderson et al., 2004; Back et al., 2005; Bang and Scott, 2003; Barreiro et al., 2004; Dando and Wagstaff, 2004; Feng et al., 2004a,b; Molina et al., 2004; Sharma et al., 2004; Wang et al., 2004).

[d] (Anderson et al., 2004; Back et al., 2005; Barditch-Crovo et al., 2001; Barreiro et al., 2004; Hawkins et al., 2005; Johnson et al., 2001; Louie et al., 2003; Murakami et al., 2004; Piliero, 2004; Robbins et al., 1998; Sharma et al., 2004; Suo and Johnson, 1998; White et al., 2002, 2005).

[e] [Ziagen package insert. GlaxoSmithkline]; (Anderson et al., 2004; Back et al., 2005; Hawkins et al., 2005; Hervey and Perry, 2000; Johnson et al., 2001; Murakami et al., 2004; Piliero, 2004; Sharma et al., 2004; Wang et al., 1999; White et al., 2005).

[f] http://www.natap.org/2004/pdf/posRep04.pdf; (Herzmann et al., 2005; Schinazi et al., 1992).

[g] http://www.natap.org/2004/pdf/posRep04.pdf; (Murakami et al., 2004; Otto, 2004; Schinazi et al., 2002; Stuyver et al., 2004; Youle and NATAP.org, 2004).

[h] (Chen et al., 1999; Furman et al., 2001; Gulick, 2003; Jeffrey et al., 2003; Kewn et al., 2003; Otto, 2004; Thompson et al., 2005).

[i] http://www.natap.org/x2004/pdf/posRep04.pdf; (Adams et al., 2004; Gu et al., 2006; Otto, 2004; Youle and NATAP.org, 2004).

[j] N/A: not available.

[k] Toxicity index is defined as the relative increase in the time required to replicate the mitochondrial genome based on the rates of incorporation and removal of chain terminators and is calculated using a formula. Please refer to text.

chain terminators. TI is calculated using the formula:

$$\mathrm{TI} = 1 + \left(\frac{k_{\mathrm{cat}}}{k_{\mathrm{exo}}}\right) \times \frac{[\mathrm{NRTI\text{-}TP}]/[\mathrm{dNTP}]}{4D},$$

where, $D = ((k_{\mathrm{pol}}/K_{\text{i-DNA pol }\gamma})\mathrm{d_{NTP}})/(k_{\mathrm{pol}}/K_{\text{i-DNA pol }\gamma})_{\mathrm{NRTI\text{-}TP}}$.

In this equation, k_{cat} represents the rate of incorporation of the natural dNTP; k_{exo} represents the rate of excision of the chain terminator; [NRTI-TP]/[dNTP] is the ratio of the cellular concentrations of NRTI-TP and natural dNTP, respectively, whereas D is the discrimination coefficient representing the ratio of specificities of the NRTI-TP and dNTP, respectively, and K_{pol} is the maximum rates of polymerization of the substrate into the mitochondrial DNA (Johnson et al., 2001; Lee et al., 2003). The values reported in Table 1 assume a [NRTI-TP]/[dNTP] ratio of 1, so that differences in mitochondrial uptake and phosphorylation are not reflected. A toxicity index of 1.0 defines zero toxicity, since the rate of replication of the genome is unaffected, while more toxic NRTI-TP have higher TI values (e.g., the TI for ddC triphosphate (ddC-TP) and d4T triphosphate (d4T-TP) are 160,000 and 3120, respectively). Although high values of TI are predictive of the *in vivo* toxicity for most NRTI, TI under-predicts the toxicity of ZDV (TI = 1.05), suggesting that factors other than inhibition of DNA polymerase gamma may be responsible for ZDV toxicity (Johnson et al., 2001). ZDV and its reduced metabolite 3′-amino-3′-deoxythymidine decrease levels of globin mRNA in differentiating K-562 leukemia cells associated with decreased globin transcription in vitro (Weidner et al., 1992; Weidner and Sommadossi, 1990). This suggests that ZDV toxicity may also be related to disruption of the regulatory processes involved in the control of gene expression during development and differentiation of erythroid cells (Weidner et al., 1992; Weidner and Sommadossi, 1990), and may produce a decrease in the binding levels of major transcription factors involved in erythroid differentiation (Bridges et al., 1996). ZDV has also been shown to specifically inhibit β-globin gene in human erythroid progenitor cells at clinically relevant concentrations (Spiga et al., 1999). Our laboratory prefers determining mitochondrial toxicity using a cell-based assay over 14 days since in general nucleoside analogs (or NRTI-TP) require transport into the mitochondria and then phosphorylation to inhibit mtDNA synthesis (Bridges et al., 1999; Chen and Cheng, 1992; Stuyver et al., 2002).

3. NRTI for first line therapy

ZDV was the first antiretroviral drug tested in the clinic and was initially used as monotherapy. Clinical trials using combinations of two NRTI, e.g., ZDV with ddC, ddI or 3TC, demonstrated a more sustained antiviral and immunological response than ZDV alone (Delta Coordinating Committee, 2001; Hammer et al., 1996; Kuritzkes et al., 1996; Schooley et al., 1996; Staszewski et al., 1996). TK1 is the primarily enzyme responsible for ZDV phosphorylation to ZDV-monophosphate (ZDV-MP). However, mitochondrial thymidine kinase (TK2) has also been shown to activate ZDV in cultured monocytes/macrophages that do not express TK1, but to a much lesser degree (Arner et al., 1992; Eriksson et al., 1989, 1991; Furman et al., 1986). Since TK1 is cell cycle dependent, ZDV is phosphorylated to a much greater extent, and is more potent in activated than in resting cells. The TK1 activity in the bone marrow of rats followed a circadian pattern, and ZDV toxicity varied with time of administration (Zhang et al., 1993a,b). However, this has not been demonstrated in humans. Thymidylate kinase catalyzes the subsequent phosphorylation to ZDV-diphosphate (ZDV-DP) and is rate limiting with a K_{m} of 12 μM versus ZDV-MP (Furman et al., 1986; Lavie et al., 1997). ZDV treatment is limited by its toxic side effects in bone marrow cells which are manifested by anemia and neutropenia in about 30% of individuals and is partially dose dependent (Collier et al., 1990; Richman et al., 1987; Spiga et al., 1999). This toxicity may be partially related to inhibition of mitochondrial DNA polymerase gamma, the sole enzyme responsible for the base excision repair of oxidative damage to mitochondrial DNA. In addition, recent studies by McKee et al. demonstrated that ZDV is a potent inhibitor of thymidine phosphorylation in heart mitochondria (Johnson et al., 2001; Martin et al., 2003; McKee et al., 2004). Thus, the toxicity of AZT in some tissues may be mediated by disrupting the substrate supply of thymidine triphosphate (TTP) for mitochondrial DNA replication (McKee et al., 2004).

The current dose for ZDV for treating HIV infections is 300 mg bid. However, a study by Barry et al., demonstrated that when the ZDV dose was lowered from 300 mg bid to 100 mg tid, the levels of active ZDV triphosphate (ZDV-TP) levels (related to antiviral efficacy), remained similar, while plasma concentrations of ZDV and intracellular ZDV-MP, associated with toxicity, varied linearly with dose (Barry et al., 1996). This result supports the possibility that the TK1 is super-saturated at ZDV doses used in the clinic (K_{m} for TK1 = 12 μM), and suggests that doses may be reduced without compromising efficacy (Furman et al., 1986). However, a recent study using ZDV at 600 mg once daily produced lower virus depletion than the 300 mg bid ZDV dose (Ruane et al., 2004), consistent with the short intracellular $t_{1/2}$ (3–4 h) of ZDV-TP in primary human PBM cells (Sharma et al., 2004), and the probable super-saturation of the TK1 kinase enzyme using this regimen. Super-saturation of TK1 would produce relatively lower ZDV-TP levels compared to the bid dose. The kinetics of phosphorylation for other NRTI discussed in this review are not saturated at clinically relevant doses. In contrast to the other NRTI mentioned, ZDV is eliminated primarily through the urinary tract in the inactive glucuronide (GAZT) form. GAZT is produced in the liver by glucuronidation by the enzyme human UDP-glucuronosyltransferase 2B7 (UGT2B7), and approximately 74% of the absorbed dose may be recovered in urine in that form (Barbier et al., 2000; Collins and Unadkat, 1989). Therefore, the clearance of ZDV may be reduced in the presence of other drugs that undergo significant glucuronidation (e.g., probenecid and sulfamethoxazole), resulting in increased plasma levels of ZDV (Morse et al., 1990). The first generic formulation for ZDV was approved in September 2005 by the US FDA. Pharmacokinetic and efficacy parameters for ZDV are summarized in Table 1 (Anderson et al., 2004; Barreiro et al., 2004; Cannard et al., 1998; Reardon and Miller, 1990; Wang et al., 1999). The population pharmacokinetics of ZDV have been

reviewed elsewhere (Burger et al., 1994; Gitterman et al., 1990; Zhou et al., 1999).

The US FDA approved 3TC, marketed by GSK/Shire, for HIV- and HBV-infected persons. It is the first "L-nucleoside" and one of the most widely used nucleoside in HAART. 3TC is an important component of fixed nucleoside combinations including, Combivir® (containing 3TC and ZDV), Epzicom® (ABC and 3TC) and Trizivir® (ABC, 3TC and ZDV). Pertinent clinical pharmacokinetics of 3TC is summarized in Table 1 (Anderson et al., 2004; Barreiro et al., 2004; Johnson et al., 1999; Wang et al., 1999) and the population pharmacokinetics of 3TC have been described elsewhere (Moore et al., 1999a). 3TC has been approved for once- or twice-daily dose regimens (DeJesus et al., 2004; Sension et al., 2002). Unlike ZDV, the phosphorylation of 3TC to the 3TC triphosphate (3TC-TP) is not saturated at serum concentrations observed in the clinic and its phosphorylation is independent of position in the cell cycle. Furthermore, 3TC may produce higher ddNTP/dNTP ratios in resting cells, which may result in greater efficacy in non-stimulated lymphocytes (Gao et al., 1994). A major disadvantage of 3TC is the rapid development of resistance *via* a single point mutation (M184V), and resistance is likely to develop after only a few weeks of monotherapy (Eron et al., 1995). However, the M184V point mutation may be exploited in HAART combination therapy, since it may increase the susceptibility of certain resistant viruses to ZDV in some persons (Nijhuis et al., 1997), and may impair viral fitness (Miller et al., 2002). Therefore, 3TC is likely to continue to play an important role in many once-daily combinations. The anti-HBV activity of 3TC is also limited by a relatively rapid development of resistance which increases with the duration of treatment (Dienstag et al., 1999a,b; Dore et al., 1999; Stuyver et al., 2006).

FTC may exist as either the (+) of (−)-enantiomer. FTC in the (−)-enantiomer form is currently marketed by Gilead Sciences for treating HIV infections either alone (Emtricitabine®) or in combination with TDF in a fixed dose combination (Truvada®). Pharmacological parameters for FTC are summarized in Table 1 (Anderson et al., 2004; Bang and Scott, 2003; Barreiro et al., 2004; Molina et al., 2004). Mechanistic studies indicated that the triphosphate of (−)-FTC (FTC-TP) is a more potent inhibitor of HIV-1 RT than 3TC-TP (Feng et al., 1999; Ray et al., 2002, 2003). Testing of the resolved enantiomers has revealed (+)-FTC to be nontoxic and approximately 10- and 40-fold less potent inhibitor of HIV and HBV replication, respectively, in cell culture, than (−)-FTC (Doong et al., 1991; Furman et al., 1992; Schinazi et al., 1992). The K_i values of the (−)- and (+)-enantiomers of FTC-TP are 2.1 and 8.6 μM, respectively, versus purified HIV-1 RT (Feng et al., 1999; Schinazi et al., 1992). (+)-FTC, but not the (−)-enantiomer may be deaminated by cytidine deaminase to the nontoxic inactive metabolite (+)-β-2′,3′-dideoxy-5-fluoro-3′-thiauridine [(+)-FTU]. The extent of deamination to (+)-FTU varies between species according to enzyme levels and substrate specificity of this enzyme (Hurwitz et al., 2005). (−)-FTC was stable to deamination, regardless of the mammalian species tested, although some deamination has been observed by bacterial cytidine deaminase (Furman et al., 1992). The 50:50 racemic mixture of the (−)- and (+)-enantiomers of FTC (Racivir) is being developed by Pharmasset Inc., and is currently in Phase 2b clinical trials (Darque et al., 1999; Furman et al., 1992; Korba et al., 2000). Neither rats, rabbits, dogs nor rhesus monkeys was able to predict the pharmacokinetics of RCV reliably in humans. However, interspecies scaling was possible using a combination of animals (Cui et al., 1996; Frick et al., 1993, 1994; Hurwitz et al., 2005; Moore et al., 1997; Schinazi et al., 1992). A small phase Ib/IIa study was performed in which RCV was administered once daily orally to HIV-1-infected treatment-naïve male volunteers for 14 days at doses between 200 and 600 mg, in combination with stavudine and efavirenz. RCV was well tolerated, and the pharmacokinetics remained linear in that dose range (Herzmann et al., 2005). Viral loads dropped in all dosage groups, with mean maximal reductions of 2.1–2.6 $\log_{10}$ below baseline on day 28, and remained suppressed for more than 2 weeks following the last dose. Virus loads remained more than 1 $\log_{10}$ below baseline levels 21 days after the last dose (Herzmann et al., 2005). Studies performed *in vitro* suggest that RCV like (−)-FTC and 3TC selects for M184V, but at a slower rate. Furthermore, RCV inhibited the development of the T215Y resistance mutation that is associated with resistance to antiretroviral agents such as ZDV and D4T (Schinazi et al, 1997). Therefore, treatment regimens with RCV may offer some advantages over (−)-FTC in the clinical setting and could prove efficacious against viral mutants containing the M184V/I mutations. Results from a clinical study testing this hypothesis are currently underway.

TDF is an ester of the acyclic adenine nucleotide phosphonate, and was developed to improve the poor oral absorption of TFV (9-[2-(phosphonomethoxy)propyl]adenine), also known as PMPA (Lyseng-Williamson et al., 2005). Oral dosing of TDF results in approximately 25–30% bioavailability measured as circulating TFV concentrations. The pharmacokinetics of TFV is dose-proportional and similar in healthy volunteers and HIV-infected individuals. The oral bioavailability of tenofovir is enhanced by administration with a high-fat meal, but is similar at steady state when administered with or without a typical meal (Barditch-Crovo et al., 2001; Kearney et al., 2004). The population pharmacokinetics of TDF have been described elsewhere (Jullien et al., 2005b). Pharmacokinetic and efficacy parameters for TDF are summarized in Table 1 (Louie et al., 2003; Robbins et al., 1998; Suo and Johnson, 1998; White et al., 2002). Since TDF already contains a phosphate group it bypasses the need for an initial potentially rate limiting monophosphorylation and is readily metabolized to the active metabolite TFV-diphosphate (TFV-DP) in cells. Due to the longevity of the intracellular TFV-DP, TDF is administered once daily. TDF is also marketed as a once-daily co-formulation pill called Truvada® that contains 300 mg of TFV (equivalent to 245 mg of TFV) and 200 mg of FTC (Dando and Wagstaff, 2004).

ABC is a structural analog of 2′-deoxyguanosine. ABC has a more complex phosphorylation pathway than other NRTI. The initial phosphorylation step involves conversion to ABC monophosphate (ABC-MP) by adenosine phosphotransferase, followed by enzymatic deamination to carbovir monophosphate (CBV-MP), which then undergoes two additional enzymatic phosphorylation steps to form the active carbovir triphosphate

(CBV-TP) by cellular kinases (Daluge et al., 1997; Faletto et al., 1997). Phosphorylation is not saturated at clinical doses of ABC. CBV-TP competes with 2′-deoxyguanosine triphosphate (dGTP) for viral RT and has a K_i of 21 nM (Daluge et al., 1997; Faletto et al., 1997). Pertinent pharmacokinetic and efficacy parameters for ABC are summarized in Table 1 (Anderson et al., 2004; Hervey and Perry, 2000; Wang et al., 1999; White et al., 2005) and population pharmacokinetics are described elsewhere (Capparelli et al., 2005; Jullien et al., 2005a; Weller et al., 2000). Preliminary results reported from a pharmacokinetic study in 20 individuals taking 300 mg of ABC bid indicate that the cellular concentration of CBV-TP remained approximately two-fold higher than the K_i value for CBV-TP versus HIV-1 RT for 24 h post-dosing. Therefore, it was concluded that once-daily dosing of ABC is a viable option (Piliero, 2004). Preliminary results from other studies also suggest that ABC is effective at 300 mg bid and once-daily regimens when combined with 3TC (Goedken and Herman, 2005; Ruane and DeJesus, 2004).

4. Fixed dose co-formulation of NRTI

The development of fixed dose co-formulations of HAART drugs are desirable since they are convenient to take and simplify compliance and limit insurance co-payments. Co-formulations of NRTI include Truvada®, Combivir® and Epzicom®. Recent data indicate that Truvada in combination with Sustiva® (STV, Efavirenz) was superior to Combivir® with STV (Gallant et al., 2006). A triple drug co-formulation containing FTC, TDF and STV is also under development. However, studies of a fixed dose triple nucleoside combination regimen containing ABC, 3TC and ZDV (Trizivir®) demonstrated the onset of a more rapid virologic failure than regimens that include a PI or an NNRTI (Gulick et al., 2004).

5. NRTI developed for second line HAART

The emergence of resistant HIV strains during therapy has made it a major challenge to develop regimens that delay, prevent or attenuate the onset of resistance. Since the dose of antiretroviral agents like 3TC are generally lower for HBV than HIV, it may be prudent to use anti-HBV agents that are not active against HIV in HBV positive individuals that are at risk for becoming infected with HIV, to avoid development of resistant HIV that may result from exposure of the virus to sub-therapeutic doses. Common resistance mutations, including TAMs, K65R and M184V, need to be a continued focus in HIV drug development. The emergence of mutations observed following exposure to the various NRTI discussed in this review are summarized in Table 1.

RVT, AVX-754 and AMDX are NRTI under development for use as second line therapies for individuals infected by viruses containing some of the common mutations. RVT, a 2′-deoxycytidine analog, is currently in Phase 2b clinical trials for the treatment of HIV infections by Pharmasset Inc. (Cohen et al., 2005). Preclinical studies indicate that RVT triphosphate (RVT-TP) had a long intracellular $t_{1/2}$ in the range of 13–17 h and inhibits replication of both wild type and mutant strains of HIV commonly observed during treatment with ZDV, 3TC and other NRTI (Schinazi et al., 2002). No mitochondrial toxicity or increase in the lactic acid was noted in human liver cells exposed for 14 days to the RVT. In 24 treatment-naïve patients, once-daily doses of 50, 100 and 200 mg as monotherapy induced a drop in viral load of approximately 1.77 logs after 10 days. Eighty-eight percent of subjects treated reached < 400 copies/ml. A recent Phase 2b clinical study demonstrated that RVT is a powerful drug against resistant viruses and it appears that no resistant viruses associated with RVT were selected (Erickson-Viitanen et al., 2006). Interestingly, RVT (200 mg dose, po) was highly effective in drug-experienced individuals that were not taking 3TC or FTC (mean reduction in viral load on week 16 was 1.4 and 1.5 $\log_{10}$) in optimized and non-optimized regimen, respectively, but was less effective in the presence of these oxathiolane cytosine analogs. Clinical parameters for RVT are summarized in Table 1 (Stuyver et al., 2004; Youle and NATAP.org, 2004). Recently, a phase 2b study was stopped due to elevated levels of lipase which could lead to pancreatitis in persons taking 200 mg RVT.

9-(Beta-D-1,3-Dioxolan-4-yl)guanine (DXG) is a guanosine nucleoside with potent and selective activity against HIV-1, HIV-2 and HBV. The key pharmacological parameters of DXG are summarized in Table 1 (Gulick, 2003; Jeffrey et al., 2003; Kewn et al., 2003). DXG triphosphate (DXG-TP) is also a potent inhibitor of wild type and drug-resistant forms of HIV-1 RT including enzymes containing M184V/I, TAMs and the 69SS double insert, and of HBV in human cells (Chin et al., 2001; Lennerstrand et al., 2005; Seigneres et al., 2002; Ying et al., 2000). Resistance develops slowly *in vitro*, and is associated with mutations at K65R or L74V. However, DXG possesses limited aqueous solubility. AMDX (DAPD) is a 2,6-diaminopurine prodrug of DXG undergoing phase 2b clinical testing by RFS Pharma, LLC (Corbett and Rublein, 2001). AMDX is rapidly absorbed orally and is deaminated by adenosine deaminase (ADA) to DXG, before undergoing intracellular phosphorylation to DXG-TP (Chen et al., 1999; Cheng and Borroto-Esoda, 2004; Feng et al., 2004b). A study with 90 HIV-1 infected individuals receiving oral doses of AMDX between 25 and 500 mg bid and 600 mg qd (DAPD-101), demonstrated approximately linear pharmacokinetics with plasma DXG concentrations remaining higher than the EC_{50} value determined *in vitro*, for the entire dosing interval, for doses greater than 100 mg twice daily (Thompson et al., 2005). Peak plasma concentrations of AMDX and DXG were observed between 1 and 2 h post-dosing and DXG plasma concentrations were much higher than those of AMDX (AUC ratios, 5–9). Mean terminal $t_{1/2}$ values for AMDX and DXG were 1–2 and 4–7 h, respectively. AMDX at 500 mg bid for 5 days produced a median reduction in plasma HIV-1 RNA of 1.3 $\log_{10}$ copies/ml in treatment naïve subjects, while HAART-experienced subjects had a median reduction in viral load was 0.7 $\log_{10}$ copies/ml (Thompson et al., 2005). HIV-1 isolated from subjects failing ZDV and 3TC therapies, remained sensitive to DXG in cell culture assays using isolated primary human PBM cells (Gu et al., 1999a,b; Mewshaw et al., 2002). A recent study indicated that AMDX was also effective in individuals that had failed in triple therapy that had a mean of six mutations in the *pol* region (Margolis et al., 2006).

AVX754 is another 2′-deoxycytidine analog with a similar structure to 3TC and FTC that is being developed by Avexa Ltd (Adams et al., 2004; Bethell et al., 2005; Taylor et al., 2000). AVX-754 has *in vitro* activity against a broad spectrum of thymidine associated mutations (TAMs) and is reported to be active against ZDV and 3TC-resistant virus *in vitro*, with a two-fold increase in the median effective concentration (EC_{50}) when mutations at M184V and codon 215 were present. The susceptibility of HIV to the drug is also reduced two-fold by the presence of up to five TAMs (Bethell et al., 2003). Resistance is slower to develop in cell culture than 3TC, and like 3TC is associated with changes at codons K65R, V75I and M184V, making it potentially cross-resistant. Viruses containing these mutations may be up to four-fold less sensitive to AVX-754. A Phase 2a clinical trial in 63 HIV-infected individuals naïve to previous drug therapy demonstrated a statistically significant >1.4 $\log_{10}$ decrease in viral load. Furthermore, AVX754 was well tolerated and demonstrated good oral bioavailability and could be used in once-daily regimens (Gu et al., 2006; Otto, 2004).

6. Novel NRTI under pre-clinical development

APD is a very soluble prodrug of DXG in preclinical development. APD differs from DAPD since it requires oxidation by xanthine oxidase in the liver for activation. The oral pharmacokinetic of APD have been studied in mice and rhesus monkeys (Chen et al., 1996; Manouilov et al., 1997). APD demonstrated rapid oral absorption, and was rapidly converted to the active DXG metabolite. The terminal half-life of APD in plasma is two-fold to four-fold shorter than DXG (produced from APD) in both species (Chen et al., 1996; Manouilov et al., 1997). Furthermore, APD produced a high DXG AUC value in all treated animals. The bioavailability of APD was 41% in mice. High plasma concentrations of DXG were observed in monkeys despite low levels of the parent drug, suggesting a significant first-pass metabolism of APD. However, the oral bioavailability of APD was not calculated in monkeys, since no iv dose was administered. Further studies are warranted for this agent, which may be developed for HIV and HBV infections by RFS Pharma LLC.

DOT, a thymidine derivative is in the preclinical stage of development. This compound seems to have all the advantages of ZDV without the bone marrow toxicity and is also effective in the presence of important TAMs (Lennerstrand et al., 2006). Oral bioavailability in rats and monkeys is close to 100% (Schinazi et al., 2005). DOT has an EC_{50} of $0.43 \pm 0.07\ \mu M$ in primary human PBM cells and is significantly active against all of the commonly found NRTI-resistant HIV-1 mutants such as: D67N, K70R, T215Y, K219Q, K65R, and M184V (Chu et al., 2005).

7. Conclusions

Data contained in this review suggest that the field of nucleoside chemistry and biology has produced numerous highly effective agents for the treatment of HIV and HBV that have prolonged the lives of millions of infected persons. An "ideal" NRTI would demonstrate all or most of the following properties: have excellent oral bioavailability, and be a good substrate for cellular kinases for conversion to the active NRTI-TP while not competing for phosphorylation with other NRTI given concurrently. The NRTI-TP should have high affinity for the HIV RT enzyme (low K_i), and low affinity for human mitochondrial DNA polymerase, no mtDNA toxicity in cell systems on prolonged treatment, no human bone marrow toxicity, have a long intracellular half-life and an excellent pharmacological profile, including transport to various reservoirs where HIV is present. Furthermore, viral resistance to the NRTI should be rare and difficult to emerge. Although no "ideal" NRTI exists yet, data contained in this review suggest that the field of nucleoside chemistry and biology is expanding and that many of these goals have been reached. The continued fine-tuning of NRTI chemistry and biology should result in molecules that have the majority of the ideal characteristics described above, and these should provide additional treatment options for persons infected with HIV.

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