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Review

Antiretroviral activity of emtricitabine, a potent nucleoside reverse transcriptase inhibitor

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Introduction

Potent triple combination regimens have greatly reduced the clinical progression, opportunistic infections and mortality associated with HIV infection [1,2]. The success of combination regimens and the need for long-term adherence to treatment have led to the search for more potent, and less toxic antiretroviral agents, more patient-friendly regimens and newer drug classes. Meanwhile, nucleoside reverse transcriptase inhibitors (NRTIs) continue to be an essential component in almost all HIV regimens [3]. An optimal combination of NRTIs appears to be essential for the inhibition of viral replication and the ultimate success of a regimen. NRTIs vary greatly in potency, side-effects, resistance profiles and dosing convenience [3]. Because the clinical utility of combination antiretroviral regimens is limited by the prevalence of undesirable side-effects, difficulty in adherence to complex dosing schedules and the emergence of viral resistance, the search for potent, safe and well-tolerated NRTIs with favourable dosing schedules remains critical.

Overview

Oxathiolane-cytosine analogues are a novel class of NRTIs that potently and selectively block HIV [4,5] and hepatitis B virus (HBV) replication [5]. Lamivudine has been approved for these indications, and emtricitabine (formerly FTC) is currently in advanced Phase III clinical trials.

The oxathiolane-cytosine class of NRTIs, particularly the newest member, emtricitabine, has shown a high degree of selectivity for human retroviruses [4,5]. In sub-micromolar concentrations, emtricitabine demonstrated antiviral activity against laboratory-adapted strains of HIV-1 and HIV-2 in various cell systems [6]. Emtricitabine also exhibits antiviral activity in cell culture against feline and simian immunodeficiency viruses (SIVs), animal lentiviruses related to HIV [4], and against HBV [5], for which it is being developed clinically.

Like other NRTIs, both emtricitabine and lamivudine are activated to triphosphate (TP) derivatives, which mediate the antiviral effect. The TPs compete with deoxycytosine TP (dCTP), the physiological substrate, to inhibit HIV-1 reverse transcriptase (RT). Both drugs lack a hydroxyl group at the 3′ position of the oxothiolane moiety [7], so incorporation of lamivudine TP or emtricitabine TP into a primer DNA strand results in chain termination, halting DNA- or RNA-directed DNA synthesis [5,8].

Potency of emtricitabine

The ability of emtricitabine to inhibit HIV replication has been compared to the anti-HIV activity of other NRTIs in a number of cell-culture systems following acute infection with various HIV-1 or HIV-2 strains.

Human T cell lines infected with a laboratory-adapted strain

Several studies have been conducted to determine the relative potency of emtricitabine in human lymphoblastoid T-cell lines (MT-4, CEM, or HT4-6C) acutely infected with a standardized infectious dose of a laboratory-adapted strain of HIV-1 (HIV-1_{IIIB} or HIV-1_{LAV}) or HIV-2 (HIV-2_{ZY}) [4,6]. Against laboratory-adapted strains of HIV-1, the EC_{50} ranges were 0.009–0.5 μM (emtricitabine), 0.005–0.06 μM (zidovudine), 0.07–3.2 μM (lamivudine), 8.5–16.0 μM (didanosine) and 0.03–0.05 μM (zalcitabine) (Table 1). Emtricitabine consistently exhibited up to 10-fold greater activity than lamivudine against all viruses tested in all T-cell lines.

Table 1. Relative activity of emtricitabine against laboratory-adapted HIV strains in human cell lines

		EC_{50} (μM)				
HIV strain	Cell line	FTC	3TC	AZT	ddI	ddC
HIV-1_{IIIB}	CEM	0.100	0.30	0.030	8.5	0.03
	MT-4	0.500	3.20	0.060	16.0	0.05
HIV-1_{LAV}	CEM	0.009	0.07	0.005	NA	NA
	HT4-6C	0.020	NA	NA	NA	NA
HIV-2_{ZY}	CEM	0.100	0.30	0.030	NA	NA
	MT-4	1.500	9.80	0.040	NA	0.04

EC_{50}, 50% effective concentration; FTC, emtricitabine; 3TC, lamivudine; AZT, zidovudine; ddI, didanosine; ddC, zalcitabine; CEM, MT-2 and MT-4, human T-lymphoblastoid cell lines; HT4-6C, CD4-expressing HeLa cell line; NA, not available. Data from Painter *et al.* [6] and Schinazi *et al.* [4].

Human PBMCs infected with a laboratory-adapted strain

Schinazi *et al.* [4,9] conducted other *in vitro* assays in human peripheral blood mononuclear cells (PBMCs) collected from healthy donors, and stimulated with the mitogen phytohaemagglutinin (PHA). EC_{50} values for each NRTI are summarized in Table 2. Emtricitabine generally demonstrated greater potency *in vitro* in human PBMCs than in MT-4 lines. This may be related to the fact that emtricitabine is phosphorylated differently in MT-4 cells versus PBMCs [4]. In human PBMCs, the activity of emtricitabine (EC_{50} 0.0007–0.01 μM) was generally greater than that of zidovudine (EC_{50} 0.004–0.025 μM) and 7- to 69-fold greater than that of lamivudine (EC_{50} 0.02–0.395 μM).

Activity against clinical isolates

Several *in vitro* studies of emtricitabine with clinical isolates of HIV-1 provide an assessment of natural variations in viral susceptibility to emtricitabine. One study used low-passage, zidovudine-susceptible clinical isolates (HIV-1_{J6} and HIV-$1_{2:DR2}$) [4]. In another study, PBMCs were collected from patients infected with HIV-1 but never treated with zidovudine [10]. Table 3 lists the EC_{50} values derived from dose–response curves quantifying the anti-HIV activity of emtricitabine, zidovudine, and lamivudine against these clinical isolates. The antiviral activity of emtricitabine (EC_{50} 0.002–0.0085 μM) was as much as 10-fold greater than lamivudine (EC_{50} 0.001–0.11 μM) and equal to or greater than that of zidovudine (EC_{50} 0.003–0.055 μM) in all isolates tested.

Table 2. Relative activity of emtricitabine against laboratory-adapted HIV strains in human peripheral blood mononuclear cells

		EC_{50} (μM)		
HIV Strain	Cell line	FTC	3TC	AZT
HIV-1_{IIIB}	PBMCs	0.0100	0.070	0.040
	Monocytes	0.0100	0.690	0.060
HIV-1_{LAI}*	PBMCs	0.0250	0.395	0.010
HIV-2_{ROD2}	PBMCs	0.0007	0.020	0.004

*K Borroto-Esoda, personal communication. EC_{50}, 50% effective concentration; FTC, emtricitabine; 3TC, lamivudine; AZT, zidovudine; PBMCs, human peripheral blood mononuclear cells. Data from Schinazi *et al.* [4], except HIV_{LAI}.

Table 3. Relative activity of emtricitabine against clinical isolates in human peripheral blood mononuclear cells

	EC_{50} (μM)		
HIV-1 clinical isolate	FTC	3TC	AZT
HIV-1_{J6}	0.002	0.01	0.05
HIV-$1_{2:DR2}$	0.002	NA	0.003
Wild type*	0.0085	0.11	0.055

*Clinical isolate from HIV-infected AZT-naive patient. EC_{50}, 50% effective concentration; FTC, emtricitabine; 3TC, lamivudine; AZT, zidovudine; NA, not available. Data from Schinazi *et al.* [4] and Mathez *et al.* [10].

Activity against drug-resistant strains of HIV

The potential for HIV-1 to develop resistance to emtricitabine or lamivudine was evaluated by serial passage of wild-type virus (HIV-1_{LAI} [11] or HIV-1_{HXB2} [12,13]) in human lymphoblastoid T-cell lines (MT-2 or MT-4) or human PBMCs and subsequent exposure to emtricitabine or lamivudine [11–13]. After exposure to either drug, resistant HIV-1 variants emerged that were over 1000-fold less sensitive to both emtricitabine and lamivudine [11,12]. Passage of HIV-1 with a combination of emtricitabine and zidovudine, however, appreciably delayed emergence of emtricitabine-resistant virus [12].

DNA sequence analysis of the amplified HIV-1 RT gene resistant to either emtricitabine or lamivudine revealed a single point mutation at codon 184 [11–13]. In both emtricitabine- and lamivudine-resistant variants, the single point mutation was primarily to valine (Met^{184}→Val; M184V) [11,12]. Emtricitabine-resistant variants showed no cross-resistance to didanosine, zalcitabine, zidovudine or the NNRTI, nevirapine [12].

Synergy with other antiretrovirals

Combination anti-HIV regimens are employed in part to limit or delay the emergence of drug resistance, as well as to improve therapeutic effect. Several *in vitro* studies have examined the effect of combining emtricitabine with another anti-HIV agent on the inhibition of HIV replication.

One study measured the effect of emtricitabine combined with either zidovudine, zalcitabine or didanosine. Each combination showed a strong synergistic effect by isobologram analysis [6]. Another study evaluated emtricitabine in combination with zidovudine, stavudine, zalcitabine or didanosine. As shown by isobologram analysis, the anti-HIV effect of emtricitabine plus zidovudine or stavudine was synergistic, whereas the effect of combining emtricitabine with

either zalcitabine or didanosine was additive [14]. A similar study evaluated emtricitabine with zidovudine, nevirapine or nelfinavir. The anti-HIV effect of combining emtricitabine was synergistic with zidovudine or nelfinavir and additive with nevirapine (P Furman, personal communication).

Mathez *et al*. [10] co-cultured PBMCs isolated from four healthy, HIV-seronegative donors with naturally infected PBMCs obtained from seven HIV-seropositive, zidovudine-naive patients. Multiple-drug effect analysis of the emtricitabine/zidovudine combination, with assumptions of mutually non-exclusive drug interactions, found a combination index of less than 1, indicating a synergistic effect [10].

Mechanistic basis of emtricitabine potency

Although results from various cell lines show the antiviral activity of emtricitabine generally to be from 4- to 10-fold greater than that of lamivudine, the molecular mechanism for this enhanced potency is not fully understood. As noted by Feng *et al*. [8], detailed comparisons of the pharmacokinetic and pharmacodynamic parameters of emtricitabine and lamivudine have not yet been conducted. The available comparative data, however, suggest that emtricitabine's enhanced potency compared with lamivudine may be due to a greater binding affinity of the TP to HIV-1 RT and a more efficient incorporation into viral DNA during RNA-dependent synthesis by HIV-1 RT [8].

Steady-state kinetic analysis of HIV-1 RT revealed no differences between emtricitabine and lamivudine that explained the more potent anti-HIV activity of emtricitabine. Indeed, the rate of phosphorylation to the active TP is similar between emtricitabine and lamivudine, and both are relatively poor substrates for degrading enzymes [8]. Kinetic analyses of enzyme reactions conducted under steady-state conditions measure the slowest step in the overall reaction and provide useful, although limited, kinetic and mechanistic information. Another method, the rapid transient kinetic approach, offers direct observation of events occurring at the enzyme active site. This approach permits the examination of the individual steps of the reaction pathway, including identification of enzyme intermediates and conformational changes that might be associated with catalysis [8].

Using the transient kinetic approach, Feng *et al*. [8] determined the rates of nucleotide binding and incorporation for the natural nucleotide, dCTP, and for the two oxathiolane-cytidine analogues, lamivudine TP and emtricitabine TP. Emtricitabine TP had a faster rate of incorporation and at least a threefold greater binding affinity for RT DNA/RNA compared to lamivudine TP. Consequently, the enzyme incorporated emtricitabine TP almost 10-fold more efficiently than it incorporated lamivudine TP. This greater incorporation efficiency and the higher binding affinity may account for the enhanced potency of emtricitabine compared to lamivudine.

Pre-clinical safety of emtricitabine

Cytotoxicity

Schinazi *et al*. [4] examined the cytotoxicity of emtricitabine, lamivudine and zidovudine in several uninfected cell cultures: T-cell lines (CEM or MT-4), PHA-stimulated PBMCs, and Vero cells (Table 4). Unlike the results for zidovudine, there was no apparent reduction in viable cells exposed to either emtricitabine or lamivudine in concentrations up to and including 100 μM.

When studied in clonogenic assays of myeloid or erythroid progenitor cells, a model for evaluating the potential for bone marrow toxicity, emtricitabine was found to be considerably less toxic than zidovudine [4].

Toxicological assessments

The pre-clinical safety assessment of emtricitabine is extensive and very favourable. Chronic toxicology studies revealed only mild, reversible anaemia limited to top doses of 3000 mg/kg/day in mice [6,15] and 500 mg/kg/day in cynomolgus monkeys [15]. Similarly, there was no indication of either maternal or fetal toxicity in reproductive and developmental toxicity studies [16].

Effect on mitochondrial function

Recent findings suggest that incorporation of an NRTI into mitochondrial DNA, especially in quiescent cells, may be one mechanism underlying the association of NRTIs with adverse events in various organ systems. Cui *et al*. [7] examined the potential for emtricitabine or lamivudine to affect mitochondrial structure and function in HepG2 cells. By all measures, no changes in mitochondrial structure or function were seen with exposure to either emtricitabine or lamivudine at concentrations up to 100 μM.

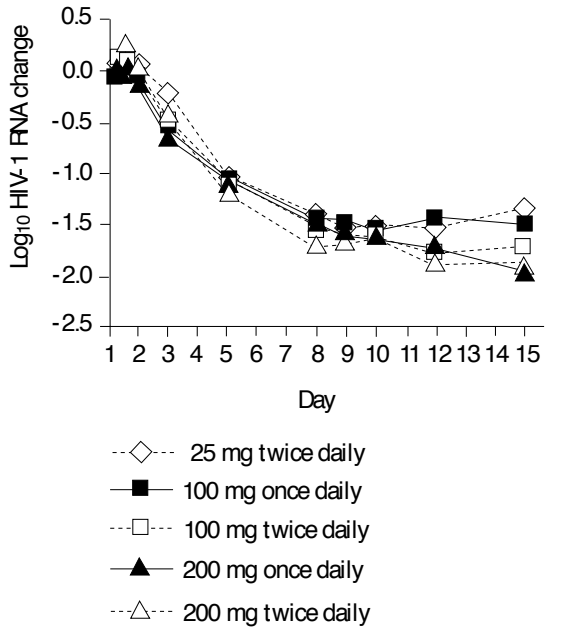
Feng *et al*. [17] recently examined the impact of

Table 4. Relative cytotoxicity of emtricitabine

	CC_{50} (μM)		
Cell line	FTC	3TC	AZT
CEM	>100	>100	14.3
MT-4	>100	>100	20.0
PBMCs	>100	>100	>100
Vero	>100	>100	28.0

CC_{50}, 50% cytotoxic concentration; FTC, emtricitabine; 3TC, lamivudine; AZT, zidovudine; CEM and MT-4, human lymphoblastoid T-cell lines; PBMCs, human peripheral blood mononuclear cells; Vero, African green monkey kidney cells. Data from Schinazi *et al*. [4,9].

Figure 1. Median viral load suppression from baseline, by emtricitabine dosing cohort



From Kahn *et al.* [19], reproduced with kind permission.

stereochemical and chemical modifications of cytidine analogues on their *in vitro* incorporation via human mitochondrial DNA polymerase (Pol γ). The TP form of a nucleoside analogue may serve as a substrate for Pol γ and result in inhibition of mitochondrial DNA synthesis. Among the cytidine analogues tested [the TP derivatives of zalcitabine, (+)lamivudine, (–)lamivudine, (+)emtricitabine and (–)emtricitabine], (–)emtricitabine TP had the lowest inhibitory effect on Pol γ, and was incorporated 24-fold less readily into the human mitochondrial DNA polymerase than was (–)lamivudine TP.

Clinical activity

The safety, pharmacokinetics and antiviral activity of a range of emtricitabine doses have been evaluated in two Phase I/II trials. Kahn *et al.* [18] hypothesized that a 14-day monotherapy regimen would be sufficient to determine the antiviral activity and intracellular pharmacology of emtricitabine, and would be adequate to define the dose for therapeutic trials. They conducted a sequential, non-randomized, dose-escalation study [18,19] in 41 HIV-infected volunteers. The doses of emtricitabine studied were 25 mg twice daily, 100 mg once daily, 100 mg twice daily, 200 mg once daily, and 200 mg twice daily.

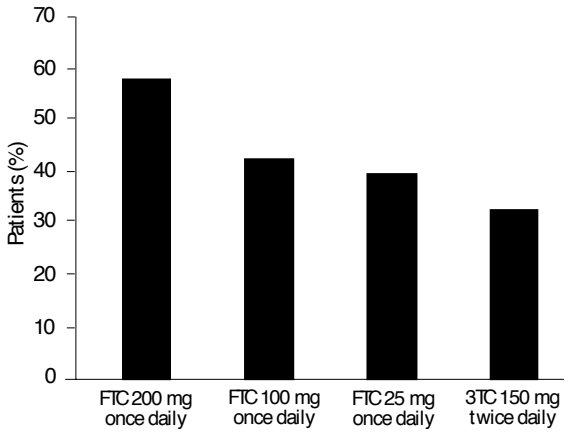
Kahn *et al.* [18,19] observed substantial reductions in HIV-1 RNA (1.72–1.92 $\log_{10}$) at a daily emtricitabine dose of 200 mg or more (Figure 1). Steady-state plasma emtricitabine concentrations were well above the IC_{90} for *in vitro* suppression of HIV-1 replication at all doses tested, and at the 200 mg once daily dose the steady-state plasma trough concentrations exceeded the IC_{90} by several fold. The plasma half-life of emtricitabine was estimated to be 7–8 h [18,19]. Steady-state intracellular emtricitabine TP concentrations increased in a dose-related manner, reaching a plateau at daily doses of 200 mg or more. Emtricitabine TP levels at day 12 of dosing were 2.5-fold higher than those observed on the initial day of dosing, which corresponds to an intracellular half-life of emtricitabine TP longer than 20 h [18].

Kahn *et al.* [18,19] reported no serious or severe adverse events related to emtricitabine, and the incidence of adverse events did not appear to be dose-related [19]. Adverse events possibly related to emtricitabine were limited to four cases of nausea, three of headache at grade 2 or more, and two each of diarrhoea, vomiting and pharyngitis [18,19]. Four of 41 patients experienced partial viral rebound, and the M184V mutant was observed in all four patients [18]. Based on virologic outcomes, plasma pharmacokinetics, and intracellular TP levels, Kahn *et al.* concluded that a single daily dose of 200 mg emtricitabine was optimal for further clinical study [18].

In a second Phase I/II, parallel-group trial, Delehanty *et al.* [20] randomized 81 antiretroviral-naive HIV-infected volunteers to receive one of three doses of emtricitabine (25, 100, or 200 mg once daily) or the standard dose of lamivudine (150 mg twice daily) for 10 consecutive days. Antiviral activity, as measured by the proportion of patients with HIV-1 RNA <400 copies/ml or a >2 $\log_{10}$ decrease from baseline, was observed in all dosing cohorts, with greater activity at the emtricitabine 200 mg once daily dose (Figure 2). Emtricitabine at 200 mg once daily also had significantly greater (P<0.05) antiviral activity than lamivudine, as measured by both average area under the curve minus baseline over the study period and slope of viral decay during the first week of treatment.

All doses of emtricitabine and lamivudine were well tolerated during this trial, and no adverse events with a severity greater than grade 2 were reported [20]. The most frequently reported adverse events that were at least possibly related to emtricitabine included headache (13%), nausea (10%), and increased appetite (7%). At the 200 mg once daily dose, one patient reported an increased appetite. No adverse events required discontinuation of emtricitabine. Results of this *in vivo* trial confirm the *in vitro* findings that emtricitabine has superior antiviral activity relative to lamivudine. Additionally, the data of Delehanty *et al.* demonstrate that emtricitabine has potent antiviral activity as a once-daily drug. Further, the data support results from Kahn *et al.* [18] that determined 200 mg

Figure 2. Proportion of patients with HIV-1 RNA <400 copies/ml or >2 log decrease from baseline



FTC, emtricitabine; 3TC, lamivudine. From Delehanty *et al.* [20], reproduced with kind permission.

once daily to be the optimal dose for future therapeutic trials.

Molina *et al.* [21,22] conducted a 48-week open-label study to assess the safety, antiviral activity and immunologic effects of a combination regimen of emtricitabine, didanosine and efavirenz administered once daily in 40 antiretroviral-naive HIV-infected patients. At baseline, median plasma HIV-1 RNA was 4.77 $\log_{10}$ copies/ml and median CD4 count was 373 cells/µl. At 24 weeks on therapy, plasma HIV-1 RNA decreased by a median of 3.5 $\log_{10}$ copies/ml, with 98% (39/40) of patients achieving plasma HIV RNA <400 copies/ml and 93% (37/40) achieving <50 copies/ml [22]. At 48 weeks, 95% (38/40) of patients maintained a plasma HIV RNA below 400 copies/ml [21]. The median increase in CD4 count was 159 and 205 cells/µl at weeks 24 and 48, respectively [21,22].

This once-daily triple combination regimen was generally well tolerated. The most common treatment-related adverse events occurred during the first 24 weeks of the study and were mild-to-moderate central nervous system symptoms (29/40, 73% of patients), diarrhoea (13/40, 33% of patients), rashes (4/40, 10% of patients) and biochemical abnormalities. Only two patients had severe hypertriglyceridemias that were possibly treatment related. No other treatment-related severe or serious adverse events were reported [21]. Molina and colleagues concluded that the favourable safety profile of this once-daily combination, along with the potent suppression of plasma HIV-1 RNA to unquantifiable levels in a large proportion of subjects, makes the combination of emtricitabine, didanosine and efavirenz an attractive once-a-day regimen. These results need to be confirmed in the setting of a comparative trial [22].

Conclusion

Early clinical results demonstrate favourable safety and pharmacokinetic profiles and significant antiviral activity when emtricitabine is given in a once-daily regimen. Emtricitabine presents several features that make it an ideal drug candidate for use in combination anti-HIV regimens: potent anti-HIV activity, synergy with other antiretrovirals, excellent tolerability, and an intracellular half-life supportive of once-daily dosing. Its *in vitro* potency against HIV is generally from four to 10 times greater than lamivudine. The potency of emtricitabine may be due to the efficiency of incorporation of its TP into HIV-1 RT. Such potency, however, does not appear to be associated with increased toxicity in animal models, as evidenced by the unremarkable findings in toxicology studies. However, the critical information, will be the activity and adverse event profile in long-term clinical studies. The profile of emtricitabine potency and safety supports larger clinical trials, which are presently ongoing.

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