Targeting of the Purine Biosynthesis Host Cell Pathway Enhances the Activity of Tenofovir Against Sensitive and Drug-Resistant HIV-1

Alonso Heredia,1 Charles E. Davis,1 Marvin S. Reitz,1 Nhut M. Le,1 Mark A. Wainberg,2 James S. Foulke,1 Lai-Xi Wang,1 and Robert R. Redfield1

1Institute of Human Virology, University of Maryland School of Medicine, Baltimore, Maryland; and 2McGill University AIDS Centre, Lady Davis Institute, Jewish General Hospital, Montreal, Quebec, Canada

Background. Targeting host-cell pathways to increase the potency of nucleoside/nucleotide analog reverse transcriptase inhibitors (NRTIs) is an important strategy for clinical investigation. Resveratrol is a natural product that inhibits cellular ribonucleotide reductase, prolonging the S phase of the cell cycle and preferentially lowering dATP levels.

Methods. We performed in vitro evaluation of resveratrol on the antiviral activity of adenosine analog tenofovir (TFV) against sensitive and drug-resistant human immunodeficiency virus type 1 (HIV-1), from subtypes B and C, in primary cells.

Results. Resveratrol enhanced the antiviral activity of TFV by up to 10-fold and restored susceptibility of TFV-resistant viruses. Resveratrol prevented wild-type HIV-1 from developing phenotypic resistance to TFV. Notably, resveratrol enhanced TFV activity against sensitive and resistant HIV-1 from both subtypes B and C.

Conclusions. Prolonged wide-scale use of thymidine analogs in the setting of viral failure has limited the efficacy of second-line NRTI-based regimens in Africa. Moreover, the extensive use of d4T has led to high frequencies of the K65R mutation, further compromising TFV efficacy. In light of increasing resistance to commonly used NRTIs in global HIV treatment programs, targeting nucleoside biosynthesis with resveratrol, or derivatives with improved bioavailabilities, is a potential strategy to maintain, enhance, and restore susceptibility of commonly used NRTIs.

Keywords. HIV-1; antiretrovirals; drug resistance; NRTI; tenofovir; subtype C; resveratrol.

Global programs expanding access to antiretroviral treatments represent a highly successful public health intervention [1]. To date, >6 million persons living with human immunodeficiency virus type 1 (HIV-1) infection have received antiviral treatment. The continued expansion and sustainability of these important programs depend on maintaining the technical feasibility of affordable and scalable antiretroviral agents to be preserved [2]. Not all antiretroviral drugs have the necessary product profile for wide-scale application. Early programs relied heavily on the use of thymidine analogs, zidovudine (AZT) or stavudine (D4T), driven by cost considerations. In addition, in the resource-limited setting, assessment of treatment outcomes was based on the clinical definition of treatment failure. As a result, patients experienced subclinical viral failure and accumulated multiple thymidine analog mutations (TAMs) that convey drug resistance to the nucleoside/nucleotide analog reverse transcriptase inhibitor (NRTI) background of currently World Health Organization–recommended second-line regimens. As such, the second-line antiretroviral regimen efficacy is limited [3].

Targeting host-cell pathways important in nucleoside biosynthesis is a strategy of potential clinical relevance, particularly as it relates to the long-term
technical feasibility of ongoing global antiretroviral treatment programs and to maintaining efficacy of nucleoside/nucleotide analogs. Previous work has shown that targeting cellular ribonucleotide reductase, which catalyzes synthesis of dNTPs, with hydroxyurea enhances the anti-HIV activities of NRTIs, including drug-resistant strains [4–6]. However, potential toxicity has limited the use of hydroxyurea in HIV-1 patients. Safer inhibitors of ribonucleotide reductase are currently being investigated against HIV-1 [7, 8].

Resveratrol (3,4′,5-trihydroxystilbene), a natural compound from grapes and other fruits, is an inhibitor of ribonucleotide reductase that preferentially lowers dATP levels and prolongs the S phase of the cell cycle [9–11], activities that may enhance nucleoside analog utilization. Resveratrol has various potential benefits to health, but it is currently unclear whether dietary concentrations are sufficient to promote them (reviewed in [12]). Resveratrol’s presence in red wine has been proposed to explain the French Paradox, a reduced risk of cardiovascular diseases in French people despite consumption of a fat-rich diet [13]. Animal studies suggest that resveratrol administration can indeed help prevent coronary heart diseases, as well as diabetes and several types of cancer (reviewed in [14]).

We have previously demonstrated synergistic inhibition of HIV-1 by combinations of resveratrol and first-generation nucleoside analogs and restoration of drug susceptibility in isolates resistant to nucleoside analogs [15, 16]. Similar results with other nucleoside analogs have been reported by others [17]. In this study, we have evaluated the effects of resveratrol on the antiviral activity of the adenosine analog tenofovir (TFV), the most commonly used NRTI and a key drug whose sustained efficacy will be central to long-term impact of global antiretroviral treatment programs.

**METHODS**

**Viruses and Drugs**

The lab-adapted strains HIV-1 BaL and HIV-1 IIIb and the primary isolate 92BR020 (subtype B) were obtained from the National Institutes of Health (NIH) AIDS Repository. The HIV-1 molecular clone NL4-3 and its derivatives carrying reverse transcriptase (RT) sequences amplified from the plasma of patients with drug-resistant HIV-1 were obtained from Dr Robert Shafer (Stanford University School of Medicine) through the NIH AIDS Repository. Subtype C primary isolates 4742, BG05, and BG15 and their corresponding TFV-resistant mutants 4742-65R, BG05-65R, and BG15-65R were provided by Dr Mark Wainberg (McGill University AIDS Centre). For tissue culture experiments, TFV (metabolite of tenofovir disoproxil fumarate prodrug), emtricitabine (FTC), and abacavir (ABC) were obtained from the NIH AIDS Repository. Resveratrol (trans form) was from Sigma.

**Cells and Infectivity Assays**

Peripheral blood lymphocytes (PBLs) were separated fromuffy coats of HIV-1–seronegative donors (New York Blood Center) by density centrifugation over Ficoll-Hypaque. For infection, PBLs were stimulated with 2.5 µg/mL phytohemagglutinin for 3 days. Stimulated PBLs were infected by incubation with virus at a multiplicity of infection of 0.001 for 2 hours. PBLs were then washed 3 times with phosphate-buffered saline and cultured in 5% carbon dioxide at 37°C, in Roswell Park Memorial Institute 1640 medium (RPMI 1640)/10% fetal bovine serum (FBS) supplemented with 10 units/mL interleukin 2 and drugs. PBLs were seeded in 96-well flat-bottom plates at a density of 2 × 10^{5} PBLs/200 µL. After 3 days of culture, half of the medium was replaced with fresh medium containing interleukin 2 and drugs. On day 7, HIV-1 p24 antigen production in the culture supernatant was assayed by enzyme-linked immunosorbent assay (ELISA).

Monocyte-derived macrophages (MDMs) were prepared and infected with HIV-1 BaL as described [18]. Briefly, 25 × 10^{6} freshly isolated peripheral blood mononuclear cells were cultured for 5 days in T-25 flasks containing culture medium, supplemented with 20% FBS and 10% AB human serum. On day 5, nonadherent cells were removed by washing 5 times with warm RPMI 1640/10% FBS medium. Adherent cells in representative flasks were counted by trypan blue exclusion. Infection was carried out by adding virus to the flask at a multiplicity of infection of 0.002 and incubating for 3 hours. Flasks were then washed 3 times with warm RPMI 1640/10% FBS medium and cultured in RPMI 1640 containing 20% FBS plus drugs. Every 3 days, culture supernatants were collected, and fresh medium-containing drugs were added to each flask. Virus replication was assayed weekly for p24 antigen production in the supernatant.

**Selection of HIV-1 Resistance to TFV in the Presence of Resveratrol**

We evaluated the impact of resveratrol on development of HIV-1 IIIb resistance to TFV by serially passaging virus in the presence of increasing concentrations of TFV. TFV was initially used at concentrations within the 50%–90% effective concentration (EC_{50}–EC_{90}) range (2 µM). Resveratrol was maintained constant at 10 µM because earlier experiments showed this concentration maximally enhances the antiviral activity of TFV. Briefly, phytohemagglutinin-activated PBLs were infected (multiplicity of infection of 0.001) with HIV-1 IIIb and cultured at 1 × 10^{5} cells/mL under each of the following conditions: no drug, resveratrol (10 µM), TFV (2 µM), and TFV (2 µM) + resveratrol (10 µM). Virus growth was evaluated on day 6 by p24 ELISA in supernatants. On day 7, 200 µL supernatant from each culture were used to infect freshly activated PBLs (2 × 10^{5}) and cells plated at the same or double concentration of TFV...
RESULTS

Resveratrol Enhances the Antiviral Activity of TFV in Primary Lymphocytes

Resveratrol inhibition of ribonucleotide reductase preferentially reduces dATP levels [9, 10]. We reasoned, therefore, that resveratrol might enhance the antiviral activity of the adenosine analog TFV to a greater extent than those of the cytosine analog FTC or the guanosine analog ABC. To test this, we compared the effects of resveratrol on HIV-1 inhibition by TFV, FTC, and ABC. We conducted these assays in PBLs infected with HIV-1 IIIB, a lab-adapted CXCR4-tropic virus, and measured viral replication by p24 ELISA in culture supernatants on day 7 after infection. In the absence of nucleoside/nucleotide analog, resveratrol concentrations of ≤10 µM did not inhibit HIV-1 replication or cell proliferation (data not shown), in agreement with our previous studies [15, 16]. However, all tested concentrations of resveratrol significantly enhanced the antiviral activity of TFV (Figure 1 and Table 1). Resveratrol significantly enhanced the antiviral activity of FTC but only at high concentrations, whereas it did not enhance the antiviral activity of ABC. These data are consistent with preferential depletion of dATP, the natural competitor of TFV-diphosphate (TFV-DP), by resveratrol. We confirmed that resveratrol enhances the antiviral activity of TFV in real-time PCR analysis with HIV-1 primer pairs specific for R/U5 (initial region of reverse transcription) and R/gag (last region of reverse transcription). In agreement with the p24 data, resveratrol alone did not inhibit HIV-1 DNA synthesis but increased its inhibition in combination with TFV (both R/U5 and R/gag transcripts) (Figure 2). Together, these data demonstrate that resveratrol by itself does not inhibit HIV-1 but enhances TFV inhibition of HIV-1 reverse transcription.

Resveratrol also enhanced TFV inhibition of CCR5 (R5)—tropic viruses BaL (lab adapted) by 6-fold and 92BR020 (primary isolate from subtype B) by 4-fold (Figure 3A). We next evaluated resveratrol in MDMs. As in our previous studies [15], resveratrol concentrations of up to 10 µM were not toxic to MDMs and did not inhibit HIV-1 BaL (data not shown). However, resveratrol greatly increased TFV inhibition of HIV-1 BaL in MDMs (Figure 3B). A higher resveratrol enhancement of TFV antiviral activity in MDMs than in PBLs is consistent with reduced levels of dNTPs in the former cell type [19, 20]. Because TFV is becoming available in geographical areas of Africa, where subtype C is prevalent, we evaluated resveratrol enhancement of TFV in subtype C primary isolates 4742 and BG05 [21, 22]. These viruses, as do most subtype C viruses, use CCR5 as primary coreceptor [21, 22]. Resveratrol enhanced TFV against 4742 and BG05 by 3- and 10-fold, respectively (Figure 3C). Together, resveratrol enhanced TFV against R5 and X4 HIV-1, from both subtypes B and C, in primary cells.
Resveratrol Restores Sensitivity of TFV-Resistant HIV-1 to TFV

The primary mutations selected for by TFV are K65R and K70E [6, 23], which reduce binding and/or incorporation of TFV-DP [24]. Although TFV does not select for TAMs, the presence of combined type 1 TAMs (M41L, L210W, and T215Y) decrease TFV susceptibility [25]. Type 2 TAMs (D67N, K70R, T215F, and K219Q/E) also decrease TFV susceptibility, although to a lesser extent than type 1 TAMs [25]. TAMs decrease susceptibility to TFV by enhancing ATP-mediated excision [26, 27]. We reasoned that resveratrol-mediated reduction of dATP, the natural competitor of TFV-DP, could favor incorporation of TFV-DP in the mutant enzymes. To test this, we evaluated the effect of resveratrol on TFV inhibition of NL4-3 molecular clones carrying RT sequences amplified from plasma of HIV-1 (subtype B) patients with decreased susceptibility to TFV [28] (Table 2). The evaluated clones had TFV EC50s that were 2.4–3.7-fold higher than wild-type HIV-1, within ranges previously described [29]. However, resveratrol increased TFV sensitivity of all mutants, reducing TFV EC50 values 4–18-fold.

Because resistance to TFV arises more frequently in patients with subtype C HIV-1 than it does in patients with subtype B HIV-1 [30–33], we also evaluated the effect of resveratrol on TFV sensitivity of subtype C TFV-resistant viruses 4742 (65R) and BG05 (65R) [21, 22]. Resveratrol reduced resistance of both 4742 (65R) and BG05 (65R) by 13-fold (Table 2).

Mutations in RT may compromise virus replication, potentially confounding interpretation of infectivity data. Thus, we evaluated the replication kinetics of the mutant viruses in the absence of drugs (Figure 4). In 2 experiments with PBLs from different donors, all viruses had peak p24 levels on the same day (day 7 in one donor and day 9 in another). Peak p24 levels of NL4-3(65R), NL4-3 (41L, 67N, 70R, 215F, 219E, 69N), and NL4-3(41L, 67N, 210W, 215Y, 69D, 44D, 118I) were 1.65 ± 0.15-fold, 2.5 ± 0.1-fold, and 3 ± 0.45-fold, respectively, which are lower than wild-type NL4-3 (means ± standard deviations [SDs]; n = 2). Subtype C HIV-1 4742(65R) replication levels were 1.85 ± 0.05-fold (mean ± SD n = 2) lower than 4742. Subtype C BG05(65R) replicated to similar levels than wild-type BG05 (1.1 ± 0.1-fold) (mean ± SD; n = 2), although replication was slightly delayed. However, all viruses from both subtypes replicated to high levels (>30 ng/mL), indicating no severe impairment in replication. Together, these data suggest

Table 1. Fifty Percent Effective Concentrations of Tenofovir, Emtricitabine, and Abacavir Against HIV-1 IIIb, in the Absence and Presence of Resveratrol, in Peripheral Blood Lymphocytes

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TFV EC50, µM (fold)b</th>
<th>FTC EC50, µM (fold)b</th>
<th>ABC EC50, µM (fold)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>RV 0</td>
<td>0.38 ± 0.07 (1)</td>
<td>0.017 ± 0.0033 (1)</td>
<td>0.10 ± 0.02 (1)</td>
</tr>
<tr>
<td>RV 1.25 µM</td>
<td>0.22 ± 0.09* (1.7)</td>
<td>0.013 ± 0.0010 (1.3)</td>
<td>nd</td>
</tr>
<tr>
<td>RV 2.5 µM</td>
<td>0.17 ± 0.05* (2.2)</td>
<td>0.008 ± 0.0005 (2.1)</td>
<td>0.09 ± 0.02 (1.1)</td>
</tr>
<tr>
<td>RV 5 µM</td>
<td>0.11 ± 0.03* (3.4)</td>
<td>0.007 ± 0.0007* (2.4)</td>
<td>0.12 ± 0.05 (0.8)</td>
</tr>
<tr>
<td>RV 10 µM</td>
<td>0.06 ± 0.02* (6.3)</td>
<td>0.003 ± 0.0016* (5.6)</td>
<td>0.08 ± 0.03 (1.2)</td>
</tr>
</tbody>
</table>

Abbreviations: ABC, abacavir; EC50, fifty percent effective concentration; FTC, emtricitabine; HIV-1, human immunodeficiency virus type 1; nd, not done; RV, resveratrol; TFV, tenofovir.

* EC50 values are means ± standard deviations from 3 experiments.

b Fold reduction in EC50 from RV 0 cultures.

* P ≤ .05 compared with RV 0 cultures by two-tailed Student t test.
that resveratrol restores TFV sensitivity of mutant viruses from both subtypes B and C in PBLs, presumably by favoring incorporation of TFV-DP.

**Resveratrol Delays Emergence of Phenotypic Resistance to TFV**

We next evaluated whether resveratrol interferes with the development of HIV-1 resistance to TFV. We serially passaged HIV-1 IIIb in PBLs in the absence of drugs and in the presence of 10 µM resveratrol, 2 µM TFV, and 2 µM TFV plus 10 µM resveratrol (Figure 5). In agreement with the previous data, resveratrol alone did not inhibit HIV-1. Both the untreated and the 10 µM resveratrol cultures exhibited similar, parallel increases and decreases in p24 levels throughout the experiment, probably reflecting growth differences among the different donor PBLs used in serial passages. At 2 µM TFV, virus replication was low for 4 passages but rose by passage 5 and continued to rise after successive doubling of TFV concentrations at passages 5, 6, and 9. In contrast, in cultures containing 2 µM TFV plus 10 µM resveratrol, p24 levels were negligible throughout the experiment. On passage 13, viruses from the TFV alone, RV alone, and untreated cultures were evaluated for sensitivity to TFV in PBLs. HIV-1 passaged in the presence of TFV was approximately 10-fold more resistant to TFV than HIV-1 passaged in the absence of drugs (TFV EC\textsubscript{50} of 14 vs 1.3 µM). However, genotypic analysis of a partial region of the RT gene amplified from infected cells (passage 13) showed wild-type amino acid sequences at positions 65 and 70 in all cultures (untreated, RV-only treatment, and TFV-only treatment). Several reasons may account for this discrepancy (see Discussion). Collectively, our data suggest that reduction of dNTP levels by resveratrol interferes with the emergence of variants with reduced sensitivity to TFV (at least in phenotypic assays), whose RT exhibit reduced processivity and decreased efficiency of rRNA-primed (-) ssDNA synthesis at low dNTP levels [34, 35].

**DISCUSSION**

Resveratrol is a natural ingredient of the human diet that inhibits inflammation and has antioxidant activity, activities which may be especially beneficial to HIV-1 patients [36, 37]. In addition, resveratrol inhibits cellular ribonucleotide reductase [9], preferentially lowering dATP pools [10]. We demonstrate that resveratrol concentrations ≤10 µM do not inhibit HIV-1 in PBLs, but enhance the antiviral activity of TFV by up to 10-fold. In contrast, resveratrol enhancement of FTC is more modest, consistent with a reduced depletion of dCTP pools [10]. Importantly, resveratrol enhances the antiviral activity of TFV at concentrations that do not inhibit PBL proliferation, in agreement with previous work demonstrating higher antiproliferation in cancer cells (EC\textsubscript{50} < 10 µM) than in normal cells or PBLs (EC\textsubscript{50} > 10 µM) [38, 39].

Resveratrol enhancement of TFV is consistent with our previous work demonstrating enhancement of ddl, another adenosine analog. Resveratrol enhances both TFV and ddl by up to 10-fold. However, unlike ddl, which requires 3 phosphorylation steps for activation, TFV only needs to be converted to TFV-DP [40].

Resveratrol enhances the sensitivity of viruses with reduced susceptibility to TFV (K65R and TAM mutants), probably by favoring incorporation of TFV-DP at decreased dATP levels rather than by affecting excision. Restoration of TFV sensitivity is noteworthy because drug combinations containing TFV are the currently preferred NRTI backbones in therapy. The K65R mutation, associated with varying levels of resistance to TFV and all other NRTIs except for AZT, is present in 2%-5% of treatment-experienced patients with subtype B HIV-1 (reviewed in [41]). The frequency of 65R is higher (up to 69%) in

![Figure 2. Resveratrol enhances tenofovir inhibition of human immunodeficiency virus type 1 (HIV-1) DNA synthesis. Activated peripheral blood lymphocytes were infected with HIV-1 IIIb and cultured in the presence of the indicated drug concentrations. On day 3 after infection, cell lysates were prepared and subjected to real-time polymerase chain reaction amplification using R/U5 and R/gag primers for detection of early and full-length HIV-1 transcripts, respectively. HIV-1 copy numbers were corrected after sample amplification using α-tubulin primers. Data (means ± standard deviations) are from 1 representative experiment of 2 independent experiments. Abbreviations: HIV, human immunodeficiency virus; RV, resveratrol; TFV, tenofovir.](image-url)
treatment-experienced patients with subtype C HIV-1 [30–33], because copying of subtype C nucleic acid templates at RT positions 64–66 results in increased RT pausing [30], giving rise to 65R variants even before therapy [42], and because of widespread use of ddi and d4T, which can select for 65R [41], in countries where subtype C is prevalent. As drug regimens containing TFV become available in Africa and India, where subtype C HIV-1 and the 65R mutation have high prevalence rates, adjuvant therapy with resveratrol may improve both potency and durability of treatment.

In addition, resveratrol prevents the emergence of HIV-1 (subtype B) variants with decreased susceptibility to TFV, at
least as shown in drug susceptibility assays, for at least 13 passages. Our genotypic assays failed to detect the 65R or 70E resistance mutations in viruses exhibiting reduced sensitivity to TFV in PBL phenotypic assays, which might be due to differential sensitivities between assays. Alternatively, mutations in RT outside the region amplified with our PCR primers may account for the resistant phenotype [43, 44]. Although additional experiments involving higher number of passages and subtype C viruses are needed, our current data support the idea that resveratrol may increase durability of TFV-containing regimens. We hypothesize that resveratrol reduction of dNTPs will prevent, or at least considerably delay, emergence of TFV-resistant variants in both subtypes B and C. First, subtypes B and C RT enzymes with the K65R mutation have decreased processivities and diminished initiation efficiencies at low dNTP levels [34, 35]. Second, K65R mutants incorporate purine nucleotides less efficiently than pyrimidines [24], suggesting that resveratrol depletion of dATPs will further interfere with the activity of the mutant RT.

Despite its possible benefit in HIV-1 patients, there are several potential limitations with the clinical development of resveratrol. Currently there are no valid data on toxicity of the chronic intake of resveratrol in humans. In a short-term (29 days) human study, gastrointestinal discomfort/diarrhea were reported only at high resveratrol doses (2.5 or 5 g per day) [14]. In mice, resveratrol doses of up to 1 g/kg/day for 18 months were not toxic [14]. In rats, a resveratrol dose of 1 g/kg/day for the entirety of their lives starting at birth was not toxic, as measured by reduced food intake, reduced body weight, or delayed sexual maturation [14]. Currently human clinical trials on resveratrol for cardiovascular and cancer prevention are ongoing [14].

Table 2. Resveratrol Restores Tenofovir Sensitivity of HIV-1 Mutants From Subtypes B and C in Peripheral Blood Lymphocytes

<table>
<thead>
<tr>
<th>Virus</th>
<th>Geometric Mean TFV EC50, µM (95% CI)a</th>
<th>Foldb</th>
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<tbody>
<tr>
<td><strong>Subtype B HIV-1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NL4-3 (wild-type)</td>
<td>0.45 (.40–.51) [1]</td>
<td>0.07 (.03–.12) [0.1]</td>
</tr>
<tr>
<td>NL4-3 (65R)</td>
<td>1.34 (.96–1.88) [3]</td>
<td>0.34 (.25–.46) [0.7]</td>
</tr>
<tr>
<td>NL4-3 (41L, 67N, 70R, 215F, 219E, 69N)</td>
<td>1.09 (.91–1.31) [2.4]</td>
<td>0.06 (.04–.10) [0.1]</td>
</tr>
<tr>
<td>NL4-3 (41L, 67N, 210W, 215Y, 69D, 44D, 118I)</td>
<td>1.68 (1.08–2.61) [3.7]</td>
<td>0.25 (.15–.44) [0.5]</td>
</tr>
<tr>
<td><strong>Subtype C HIV-1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4742</td>
<td>1.47 (.52–4.18) [1]</td>
<td>0.08 (.02–.26) [0.05]</td>
</tr>
<tr>
<td>4742 (65R)</td>
<td>2.73 (.28–3.59) [1.8]</td>
<td>0.20 (.05–.72) [0.1]</td>
</tr>
<tr>
<td>BG-05</td>
<td>1.12 (.34–3.44) [1]</td>
<td>0.16 (.06–.40) [0.1]</td>
</tr>
<tr>
<td>BG-05 (65R)</td>
<td>4.75 (.74–10.25) [4.2]</td>
<td>0.35 (.12–.99) [0.3]</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; EC50, fifty percent effective concentration; HIV-1, human immunodeficiency virus type 1; RV, resveratrol; TFV, tenofovir.
a EC50 values determined by variable slope nonlinear regression analysis using GraphPad Prism software. Each virus was run in 3 assays, with different donors in each assay.
b Fold change from corresponding wild-type HIV-1 (NL4-3, 4742, or BG-05).

Figure 4. Replication kinetics of wild-type human immunodeficiency virus type 1 (HIV-1) and reverse transcriptase (RT) mutants, from subtypes B and C, in peripheral blood lymphocytes (PBLs). Equivalent amounts of virus (standardized to contain 10^5 50 % tissue culture infectivity dose [TCID50s]) from subtype B (A) and subtype C (B) were used to infect 10^6 phytohemagglutinin (PHA)–activated PBLs. Infected cells were cultured in interleukin 2 medium in the absence of drugs. Supernatants were harvested at the indicated times, and p24 antigen concentrations were measured by enzyme-linked immunobosorbent assay. Data are from a representative experiment; similar results were obtained in an additional experiment with PBLs from a different donor.
Another potential limitation for the clinical use of resveratrol is its low bioavailability, mainly due to inactivation and excretion by glucuronidation and sulfation, as we and others have shown [16, 45]. Most in vitro studies report resveratrol biological activity at concentrations in the 10–100 µM range [12], with our data demonstrating enhancement of TFV antiviral activity in the 1.25–10 µM range (Table 1). However, daily resveratrol administration of 5 g gives a plasma concentration of only 4.24 µM [46], which may not be sufficient for full therapeutic effects. Yet, resveratrol has demonstrated activity against cancer and cardiovascular disease in vivo [14]. The in vivo effects of resveratrol might be explained by (1) conversion of resveratrol glucuronides and sulfates back to resveratrol in liver [45]; (2) enterohepatic recirculation through biliary secretion of metabolites and subsequent deconjugation by gut microflora followed by reabsorption [47]; and (3) biologic activity of metabolites. It is possible that these metabolic pathways may increase the effective intracellular levels of resveratrol in HIV-1 patients, thereby enhancing TFV activity. In particular, conversion of resveratrol glucuronides back to resveratrol by β-glucuronidases may be especially important because β-glucuronidases are released from neutrophils during inflammation [48], a hallmark of HIV-1 infection. Similarly, ubiquitously present tissue sulfatases may contribute to convert conjugates back to resveratrol. In contrast, resveratrol metabolites are not likely to account for resveratrol anti-HIV-1 activities because our results with the main metabolites in plasma show that they neither inhibit HIV-1 nor enhance NRTIs [16].

Alternatively, resveratrol bioavailability might be improved with its naturally occurring analogs, triacetyl-resveratrol (trans-3,5,4′-triaceetylstilbene) and pterostilbene (3,5-dimethoxy-4′-hydroxystilbene), which have better bioavailabilities [49, 50], or with recently developed derivatives [17].

In summary, resveratrol (or derivatives with improved bioavailability) warrants further clinical evaluation, particularly because of its potential to secure the long-term technical feasibility of global antiretroviral treatment programs. Resveratrol could be used in combination with nucleoside/nucleotide regimen backbones for the treatment of HIV-1, enhancing NRTIs (most notably TFV), sensitizing drug-resistant strains, and preventing (or at least delaying) the emergence of drug resistance.

### Notes

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