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Short communication

The antiretroviral potency of emtricitabine is approximately 3-fold higher compared to lamivudine in dual human immunodeficiency virus type 1 infection/competition experiments in vitro

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ABSTRACT

The increasing number of antiretroviral drugs leads to mounting possibilities of combinations for the antiretroviral therapy (ART) of HIV-1 infected patients. Thus, it is of interest to determine the most potent combination of antiretroviral drugs for the first ART to delay the development of drug resistance. We have investigated the differences in the inhibitory potencies of the nucleoside reverse transcriptase inhibitors (NRTI) lamivudine (3TC) and emtricitabine (FTC) using an in vitro model based on simultaneous infection of T cells with drug-sensitive and drug-resistant viruses. Changes of frequencies in these virus populations have been measured by allele-specific real-time PCR allowing simultaneous quantification of different HIV-1 variants in the same sample. We show that the suppression of drug-sensitive viruses is significantly enhanced by FTC compared to 3TC. Mathematical modeling of the distinct rates of suppression of drug-sensitive viruses revealed an approximately 3-fold higher antiretroviral potency for FTC compared to 3TC.

Today, one of the main goals of antiretroviral therapy (ART) is long-lasting suppression of HIV-1 replication. The already high amount of different antiretroviral compounds and their increasing number is raising the question about the most potent combination useful for antiretroviral therapy, thus, avoiding or delaying the development of resistance. It is therefore important to evaluate the antiretroviral potencies of the different drugs. Comparisons of inhibitory concentrations 50% and other markers based on IC_{50}, for instance, the inhibitory quotient (IQ, trough drug level (C_{trough}) divided by the IC_{50}) (Hoefnagel et al., 2005), are commonly used as indicators for the inhibitory potency of antiretroviral drugs. However, this has lead to contradictory results with regard to 3TC and FTC, sometimes even though the same virus strain and the same source of cells were used (Boucher et al., 1993; Hazen and Lanier, 2003; Schinazi et al., 1992). Recently, it has been shown that comparisons of IC_{50} and inhibitory quotient alone might not be sufficient to determine the inhibitory potential of an antiretroviral drug and that the dose-response curve slope might be a more accurate measure of drug potency (Shen et al., 2008).

Dual HIV-1 infection/competition assays have been commonly used for the estimation of replicative capacities of different virus variants (Allers et al., 2007; Lu and Kuritzkes, 2001; Paintsil et al., 2006; van Maarseveen et al., 2006; Weber et al., 2003). Here, we describe its use for comparing the potencies of 3TC and FTC in vitro using a dual HIV-1 infection/competition model based on the assumption that more potent drugs lead to stronger inhibition of drug-sensitive viruses and at the same time to faster selection of pre-existing drug-resistant viruses (Bonhoeffer and Nowak, 1997). The suppression of drug-sensitive viruses was calculated using the model of the kinetic constant, which includes multiple, consecutive time points, thus, allowing an accurate depiction of the changes within different virus populations.

For this approach, CEMx174 cells were bulk-infected with both the drug-sensitive variant HIV-1 NL4-3 and the 3TC/FTC-resistant variant HIV-1 NL4-3_{M184V} in the estimated ratios of 9:1, 19:1, and 99:1 as previously described (Allers et al., 2007). Allele-specific real-time PCR revealed that $91.0 \pm 0.3\%$, $95.8 \pm 0.4\%$, and $99.3 \pm 0.1\%$ of drug-sensitive HIV-1 NL4-3, respectively, was used for the bulk infection, thus, almost matching the estimated ratios. Cells were equally dispersed and cultivated in different 3TC and FTC concentrations over a time period of 25 days and passaged every 5th day. 3TC and FTC susceptibility assays were performed with CEMx174

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cells to obtain IC_{50}, IC_{75}, and IC_{95} (Walter et al., 1999). Virus replication was measured by quantitative real-time PCR and virus titers increased after each passage in 35/36 cell cultures indicating that virus replication occurred and that the cell culture system was not exceeding its limits (data not shown). Allele-specific real-time PCR was performed to distinguish between the two viral variants. The limit of detection of the drug-sensitive variant HIV-1 NL4-3 is 0.1% in the M184M/V AS-PCR assay (Metzner et al., 2003).

Replication of the drug-sensitive variant HIV-1 NL4-3 in the presence of 3TC/FTC-resistant HIV-1 NL4-3 was suppressed in all 3TC-containing cell cultures. The strongest suppression of the drug-sensitive variant HIV-1 NL4-3 was achieved with 95% inhibitory concentration of 3TC, the weakest using 3TC IC_{50} (Fig. 1). As expected, the strength of inhibition was positively correlated with the inhibitory concentration, which confirms the accuracy of our model. The ratio between drug-sensitive and 3TC/FTC-resistant viruses had almost no impact on the inhibitory potency of 3TC IC_{95} on the drug-sensitive viruses. However, the replication of drug-sensitive HIV-1 NL4-3 was more efficiently suppressed at lower inhibitory concentrations of 3TC when initially 5 or 10% of the viruses were 3TC/FTC-resistant. Throughout the 25 days, the drug-sensitive variant HIV-1 NL4-3 was never completely suppressed in any of the 3TC-containing cell cultures. The percentage of HIV-1 NL4-3 was below the detection limit of 0.1% at only three time points in three different cell cultures, however, drug-sensitive viruses were detectable again at all following time points (Fig. 1). The persistence of drug-sensitive viruses in the presence of 3TC is in accordance to our previous study, where we also observed that the

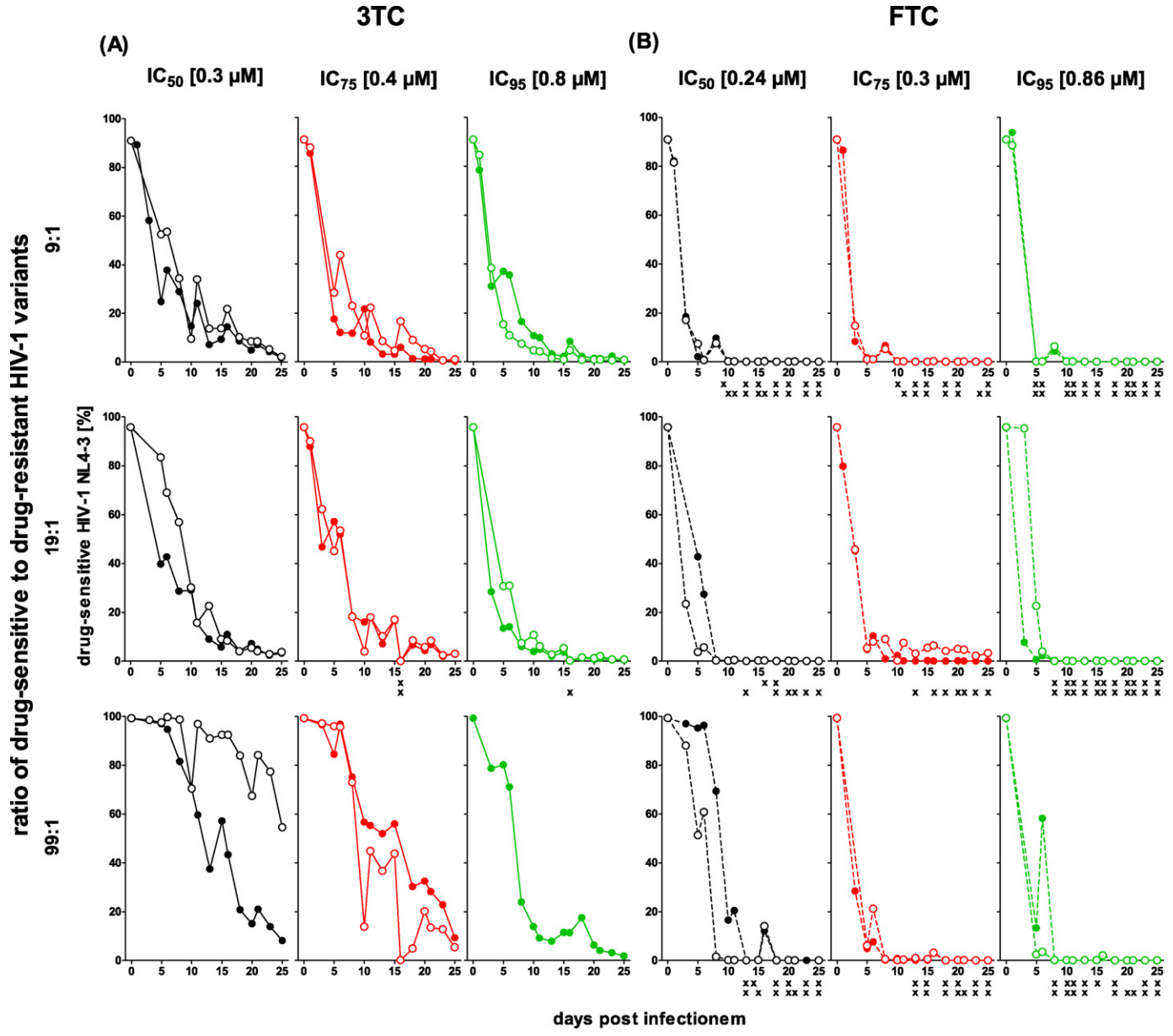

Fig. 1. Population dynamics of 3TC/FTC-sensitive HIV-1 NL4-3 in dual HIV-1 infection/competition experiments in regard to 3TC versus FTC. CEMx174 cells were infected with different ratios of HIV-1 NL4-3 and HIV-1 NL4-3_{M184V} (9:1, 19:1, and 99:1). Cells were cultivated in the presence of 3TC (A) or FTC (B) at inhibitory concentrations of IC_{50} (black), IC_{75} (red), and IC_{95} (green). Part of the cells and supernatants were passaged every 5th day. Ratios of HIV-1 NL4-3 and HIV-1 NL4-3_{M184V} were estimated by AS-PCR on days 1, 3, and 5 of each passage and on day 0 of the experiment. Percentages of HIV-1 NL4-3 are shown in filled and open circles representing two independent experiments for each set of drug concentration, virus inoculum, and drug. Standard deviations are within the data points. Crosses indicate the time points when HIV-1 NL4-3 felt below the limit of detection of the M184 M/V AS-PCR assay (0.1%). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Table 1
Differences in antiretroviral potencies of 3TC and FTC. The approximately exponential decay of the fraction of drug-sensitive virus over time is displayed as kinetic constant, which is a parameter for the differences in replication efficiencies of drug-sensitive HIV-1 NL4-3 and 3TC/FTC-resistant HIV-1 NL4-3_{M184V} viruses.

Ratio HIV-1 NL4-3: HIV-1 NL4-3_{M184V}	Kinetic constant, k (1/day)[a]					
	IC_{50}		IC_{75}		IC_{95}	
	3TC	FTC	3TC	FTC	3TC	FTC
9:1 (1)[b]	−0.13	−0.58	−0.22	−0.58	−0.19	−0.62
9:1 (2)	−0.13	−0.56	−0.17	−0.54	−0.20	−0.60
19:1 (1)	−0.14	−0.25	−0.16	−0.45	−0.19	−0.81
19:1 (2)	−0.15	−0.36	−0.15	−0.09	−0.20	−0.83
99:1 (1)	−0.11	−0.40	−0.10	−0.62	−0.15	−0.65
99:1 (2)	−0.02	−0.66	−0.13	−0.29		−0.77
Mean ± SD	−0.11 ± 0.05	−0.47 ± 0.15	−0.16 ± 0.04	−0.43 ± 0.20	−0.19 ± 0.02	−0.71 ± 0.10
p[c]	0.002		0.065		0.004	

[a] Negative values indicate a decay.
[b] Each experiment was performed in duplicates as indicated by (1) and (2).
[c] Statistical analysis was performed using the two-tailed Mann–Whitney U test for independent samples and the software Prism5 (GraphPad Software, San Diego, CA, USA). A p-value of <0.05 the difference between two groups was considered significant.

drug-sensitive and the 3TC/FTC-resistant viruses reached a certain steady state level in the presence of 3TC (Allers et al., 2007).

In corresponding dual HIV-1 infection/competition experiments using FTC, we also observed that the strength of suppression of the drug-sensitive variant HIV-1 NL4-3 was dependent on the inhibitory concentration of FTC. The initial ratio between drug-sensitive and 3TC/FTC-resistant HIV-1 NL4-3 had less impact on the inhibitory potency of FTC in our cell cultures compared to 3TC (Fig. 1). In 16/18 cell culture experiments, the drug-sensitive variant HIV-1 NL4-3 fell below the detection limit between days 5 and 13 post infectionem and was not reselected in these cell cultures. This phenomenon was never observed in any of the cell cultures containing 3TC. The phenotypic resistance profile and the biological cutoffs of 3TC and FTC are similar, thus, could not explain these differences (Borroto-Esoda et al., 2007). There is only one exception in the panel of cell cultures containing FTC: drug-sensitive viruses were able to replicate despite the presence of FTC and represented 0.3–7.5% of the virus population during the 25 days in one cell culture treated with the 75% inhibitory concentration of FTC (Fig. 1).

In our context, the kinetic constant represents the ability of a certain antiretroviral drug to inhibit drug-sensitive viruses while drug-resistant viruses are selected in the same cell culture. The kinetic constants of HIV-1 NL4-3 in our dual HIV-1 infection/competition experiments in the presence of 3TC or FTC was calculated by the formula $k = \log(1 - f(d))/d$ where d is the time, $f(d)$ is the fraction of drug-resistant virus HIV-1 NL4-3_{M184V}, $1 - f(d)$ is the fraction of drug-sensitive virus HIV-1 NL4-3, and k is the slope of the approximately straight line in the logarithmic plot of $(1 - f(d))$ over time d, i.e., a kinetic constant describing the approximately exponential decay of the fraction of drug-sensitive virus over time. Day 0 represents the ratio of HIV-1 NL4-3 and HIV-1 NL4-3_{M184V} in the virus mixture used for the bulk infection. The formula of the kinetic constant includes all percentage values of the drug-sensitive virus in the cell culture until this virus population is not detectable anymore, i.e., the lower the kinetic constant the less replication competent are the drug-sensitive viruses. Therefore, the kinetic constant can be used to express the strength of inhibition of drug-sensitive viruses by an inhibitor thereby representing a value for the potency of a specific antiretroviral drug.

The kinetic constants of drug-sensitive viruses in the presence of 3TC were decreasing with increasing drug concentrations, which represents the increment of drug potency depending on the drug concentration. This effect was considerably pronounced in regard to FTC (Table 1). Compared to 3TC in the corresponding inhibitory concentration, the kinetic constants were significantly lower in the FTC-containing cell cultures (Table 1), except for IC_{75}. Here, a significant result was not obtained due to the one FTC-containing cell culture, in which drug-sensitive viruses persisted during the 25 days of follow-up. Excluding these data, the p-value would be 0.004. The mean kinetic constants of drug-sensitive viruses in the presence of FTC are 2.7–3.8-fold higher relative to those observed in the presence of 3TC.

Shen et al. (2008) recently demonstrated that the measurement of IC_{50} and IQ is alone not sufficient to characterize the inhibitory potency of antiretroviral drugs, because these measurements do not include the dose-response curve slopes, which might reflect more accurately the inhibitory potency of an antiretroviral drug. They have shown that despite similar IC_{50} the slopes can differ. In contrast to our results, they estimated almost similar inhibitory potencies for 3TC and FTC based on slopes (m values representing log reduction in infectivity) of 1.15 ± 0.12 and 1.18 ± 0.10, respectively (Shen et al., 2008). There are some differences in the experimental designs of both studies. Shen et al. performed a single-round infectivity assay using pseudotyped viruses and we used replication competent, full-length HIV-1 allowing several rounds of infection. Both studies are based on HIV-1 NL4-3, thus, differences in the *pol* gene cannot explain the observed discrepancies. They infected primary $CD4^+$ T lymphoblasts whereas we used the T-cell/B-cell hybrid cell line CEMx174. In our previous study, we have observed, that drug-sensitive viruses persisted also in HIV-1 infected primary peripheral blood mononuclear cells (PBMC) containing 3TC and that the selection of 3TC/FTC-resistant viruses did not significantly differ in PBMC and CEMx174 cells (Allers et al., 2007). Another reason for the choice of a cell line was our observations and those of others that inhibitory concentrations 50% of 3TC and FTC can differ substantially with regard to various blood donors (Allers et al., 2007 and reviewed in Schinazi, 2003). In addition, long-term observations of changes within viral populations is almost infeasible using PBMC from the same donor.

Our dual HIV-1 infection/competition assays and the calculation of kinetic constants revealed an approximately 3-fold higher inhibitory potency of FTC compared to 3TC in agreement with clinical studies indicating that FTC is more potent than 3TC (Gallant et al., 2006; Margot et al., 2006; Rousseau et al., 2003). Dual HIV-1 infection/competition assays and the evaluation of the suppression level of certain antiretroviral drugs might be a useful tool to estimate the inhibitory potencies of antiretroviral drugs.

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