Short communication

Virological evidence supporting the use of raltegravir in HIV post-exposure prophylaxis regimens

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Background: The goal of post-exposure prophylaxis (PEP) for HIV is to prevent the establishment of a persistent infection following exposure to the virus. Integrase inhibitors have several potential advantages in PEP regimens, including the capacity to inhibit integration of HIV genomes that have already proceeded through reverse transcription, thereby becoming refractory to reverse transcriptase inhibitors. We sought to determine if integrase inhibitors extend the window of time during which PEP intervention might be successful.

Methods: Primary costimulated CD4+ T-cells or macrophages were infected with a luciferase-bearing HIV reporter virus, permitting sensitive detection of viral gene expression under different drug treatment conditions. Relevant antiretroviral agents were added at various pre- or post-infection time points.

Results: We showed that raltegravir effectively blocks HIV infection, even when cells are challenged with a large amount of virus. We also demonstrated that during infection of both primary costimulated CD4+ T-cells and primary macrophages, raltegravir can inhibit infection when added at later post-infection time points than the reverse transcriptase inhibitor efavirenz.

Conclusions: This longer post-infection efficacy window, coupled with favourable pharmacokinetic properties and low toxicity, suggest that raltegravir may prove useful in HIV PEP.

Introduction

Occupational exposure to HIV occurs in a variety of medical and laboratory settings. This may include a percutaneous injury (for example, a needlestick or cut with a sharp object), contact of mucous membranes or non-intact skin with blood, tissue or other body fluids that are potentially infectious, or accidental inoculation with high-titre virus preparations commonly used in HIV research settings. The recommended procedure following such an event is to assess the circumstances to determine the likelihood of exposure to HIV and the risks of transmission. If exposure to HIV is deemed likely, multidrug post-exposure prophylaxis (PEP) is recommended [1]. The goal of this treatment is to prevent the virus from establishing a persistent infection in the exposed individual. Unfortunately, a substantial proportion of individuals who take PEP after occupational exposures to HIV-positive sources do not complete a full 4-week course of therapy because of inability to tolerate the medications currently prescribed. Moreover, infection has been described despite PEP regimens that include drugs that inhibit the activity of the viral reverse transcriptase and protease enzymes [1].

During infection of a target cell, HIV must first reverse transcribe a DNA copy of its RNA viral genome and then integrate this DNA into the host cell's chromosome. Before integration, the HIV genome is labile and decays with a half-life of approximately 1 day [2]. However, once it has integrated, the viral genome can persist for the life of the infected cell, making it impossible to cure HIV-infected individuals with currently available antiretroviral agents [3]. Therefore, the best opportunity for preventing a persistent infection is to abort the replication cycle of HIV before it can integrate. HIV integrase inhibitors are now available, with raltegravir representing the first of these inhibitors to be approved for clinical use in adults by the US Food and Drug Administration.
Current guidelines for HIV PEP from the US Public Health Service do not offer recommendations on the use of raltegravir, because raltegravir only became available after these guidelines were developed [1]. However, raltegravir has a number of potential advantages over other antiretroviral agents. Most importantly, since raltegravir targets a later stage of the virus life cycle than reverse transcriptase inhibitors, the length of time after exposure to virus in which the drug can still prevent infection may be extended. Here, we show that raltegravir can indeed inhibit infection of primary CD4+ T-cells and macrophages when added at later post-infection time points than a reverse transcriptase inhibitor.

**Methods**

**Viruses**  
To avoid sensitivity issues associated with the use of wild-type HIV, most infections were performed using denv(Wt), which is a vesicular stomatitis virus-pseudotyped reporter virus that expresses the enhanced green fluorescent protein fused to firefly luciferase in place of Env [4]. All other HIV open reading frames are intact. To generate an integrase-deficient version of this virus, the D64E integrase mutation was introduced into denv(Wt) to produce denv(D64E) by exchanging the 2942 base pair SbfI-Sall fragment of NL(luc)AbglD64E [5] into the corresponding region of denv(Wt). Virus was produced by transfection of plasmid DNA into 293FT-cells and titred as described previously [6].

Cell isolation and infection procedures  
Prior to infection, Sup-T1 cells were treated for 24 h with the relevant antiretroviral agents. During infection, 50,000 cells were incubated for 2 h with virus at a multiplicity of infection of 2.5 in a 110 μl volume of media containing 10 μg/ml polybrene. Following infection, cells were washed and then resuspended in 200 μl of media containing the antiretroviral agents. After 2 days, cells were harvested and subjected to a luciferase assay as previously described [6].

The procedures for infection of primary CD4+ T-cells and macrophages were as described in detail elsewhere [6]. Significant differences between drug treatments at each time point were assessed using Student’s t-test.

**Results**

To determine how effectively raltegravir inhibits HIV under optimal conditions, we pretreated Sup-T1 cells with different concentrations of the reverse transcriptase inhibitor efavirenz, or one of the integrase inhibitors: raltegravir, L-870,812 or MK-2048. These drugs were then maintained throughout the subsequent culture period. Treatment with higher concentrations of each drug resulted in a substantial reduction in luciferase expression from the HIV reporter virus (Figure 1A). However, the HIV gene expression in the integrase inhibitor-treated cells remained at a higher level than for efavirenz (Figure 1A). We then directly compared denv(Wt) infection in the presence of raltegravir with infection using a virus that contains the D64E active site mutation in integrase, which prevents integration [5]. Treatment of denv(Wt)-infected cells with 10 μM of raltegravir or efavirenz resulted in a significant decrease in reporter gene activity, with efavirenz again inhibiting slightly more gene expression than raltegravir (Figure 1B). However, when an equivalent p24 input of denv(D64E) virus was used for infection, luciferase expression was almost indistinguishable from denv(Wt) infection in raltegravir-treated cells. Addition of raltegravir to the denv(D64E) infection did not affect reporter gene expression, but addition of efavirenz did suppress expression of this virus, indicating that the residual expression observed in raltegravir-treated cultures is likely due to low-level gene expression from non-integrated viral DNA, as has been previously demonstrated [7], and not a replication-competent integrated viral intermediate. Hence, even under conditions where a uniform population of highly susceptible target cells are infected at a high multiplicity of infection, raltegravir functions as efficiently as its mechanism of action allows.

We then determined whether the longer period of time between reverse transcription and integration would provide an additional window of opportunity to inhibit infection of primary cells newly exposed to the virus. To test this, primary costimulated CD4+ T-cells or primary macrophages were infected with denv(Wt) and either raltegravir or efavirenz was added at various times post-infection (Figure 2A). Both drugs inhibited infection when added at early post-infection time points (from approximately 0 h to 6 h post-infection), but at later time points raltegravir inhibited infection more effectively than efavirenz (Figure 2B–2E), particularly in monocyte-derived macrophages.

**Discussion**

Raltegravir is the first HIV integrase inhibitor approved for clinical use. Here, we show that during infection of a highly susceptible cell line, raltegravir reduces viral gene expression to the minimal level seen when HIV does not have a functional integrase gene (Figure 1). Therefore, even under exposure conditions where highly susceptible cells are infected with a large amount of virus, such as might occur following exposure of laboratory workers to concentrated HIV samples, raltegravir performs as effectively as its mechanism of action allows.

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A key consideration for HIV PEP is the post-exposure timeframe during which therapy is initiated. Current guidelines suggest that PEP should be initiated as soon as possible following exposure, ideally within hours [1]. However, the time before administration may be delayed depending on the particular circumstances, including the availability of medical advice and appropriate antiviral drugs. In this study we found that raltegravir inhibited significantly more infection than efavirenz when added at later time points (Figure 2). The fact that macrophage infection was also effectively inhibited in this way is of considerable interest because CCR5-tropic HIV strains, which are capable of infecting macrophages, are the most commonly transmitted HIV variants. The relatively short period of time between reverse transcription and integration identified here and in a related study [8] is unlikely to be responsible for the different viral load decay kinetics identified in patients treated with raltegravir versus efavirenz-containing antiretroviral regimens [9]. However, this several hour difference may be critical in the context of HIV PEP, where the goal is to prevent establishment of the initial infection.

Although protease inhibitors have been used in PEP, they only function after the virus has integrated and started producing new virions, and therefore these inhibitors may still allow early formation of a stable integrated HIV reservoir. Moreover, protease inhibitors have been reported to function suboptimally in chronically infected macrophages [10].

Additional reasons that raltegravir may prove beneficial in PEP include its rapid absorption, median time to peak plasma concentration in healthy fasting volunteers ranging from 0.5 to 1.3 h, and tolerability [11]. In view of the safety, efficacy and limited interactions with other antiretroviral drugs, raltegravir is now included as part of a preferred regimen for treatment-naive patients [12]. Furthermore, raltegravir is unaffected by mutations that confer resistance to other classes of antiretroviral drugs [13].

Further pertinent information is provided by recent case reports describing the use of raltegravir in PEP regimens for three individuals [14,15]; raltegravir was well-tolerated in each case. Promising results were also reported in a clinical trial [16] assessing the safety, adherence and tolerability of raltegravir use in conjunction with tenofovir disoproxil fumarate and emtricitabine in the context of HIV PEP. Moreover, raltegravir was shown to be capable of preventing mucosal HIV transmission in a humanized mouse model when administered as PEP [17].

In summary, we found that raltegravir can inhibit infection of primary cells when added at later post-infection time points than a potent reverse transcriptase inhibitor. Coupled with its low in vivo toxicity, good
Figure 2. Post-infection drug addition time course

(A) Diagram showing procedure for time course experiment using primary macrophages and CD4+ T-cells. In brief, cells were isolated by negative (T-cells) or positive (monocytes) immunomagnetic separation (Miltenyi Biotec, Bergisch Gladbach, Germany) from peripheral blood mononuclear cells obtained from healthy donors. CD4+ T-cells were costimulated via ligation of CD3 and CD28 in the presence of 20 units/ml of interleukin-2. Monocytes were differentiated into macrophages in the presence of 10 ng/ml of macrophage colony stimulating factor. Cells were infected and then drug was added at various post-infection times. (B) CD4+ T-cells were infected and a 10 μM final concentration of drug was added at the indicated times. At day 3 post-infection the cells were assessed for reporter expression levels. (C) Macrophages were infected and 10 μM of drug was added at the indicated time. Cells were harvested and luciferase reporter expression assessed at day 5 post-infection. Error bars represent the mean ± se, n=4. *P≤0.05 (Student’s t-test). (D) and (E) represent autologous cells from a different healthy donor treated as described for panels (C) and (D). EFV, efavirenz; RAL, raltegravir.
pharmacokinetic/pharmacodynamic properties and lack of cross-resistance with other classes of antiretroviral agents, these data suggest that raltegravir may be a useful addition to PEP regimens.

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MDM and JAZ conceived the study. MDM performed the experiments. MDM, PAK, and JAZ analysed the data and wrote the manuscript.

Disclosure statement

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