



Evaluation of the Lymphocyte Trafficking Drug FTY720 in SHIV-infected Rhesus Macaques

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ABSTRACT

Background

FTY720 is a drug that causes retention of lymphocytes in lymphatic tissues, resulting in the depletion of lymphocytes from blood and peripheral tissues. Previous studies have shown that administration of FTY720 during chronic LCMV (Lymphocytic Choriomeningitis virus) infection of mice can decrease and even eliminate virus burden. We here address the hypothesis that therapeutic use of FTY720 in SHIV-infected rhesus macaques could also lead to a decrease in viremia.

Methods

FTY720 was administered intravenously to three SHIV_{SF162P2}-infected rhesus macaques at 39, 7 or 6 weeks of infection; 3 additional control macaques (7, 48 or 6 weeks of infection) did not receive drug. Because an effective dose has not been reported in rhesus macaques, FTY720 was first given at 0.004 mg/kg on days 0, 1, 2, 14, 15, 16, followed by 0.1 mg/kg on days 28, 29, 30, 42, 43 and 44. Blood was collected 8 times throughout and 4 times during 47 days following the last injection. Plasma virus load, proviral DNA, and CD4⁺ T cell numbers were analyzed. Statistical significance was determined by unpaired t-test of mean data and the standard error of the mean.

Results

Only the 0.1 mg/kg FTY720 dose resulted in a statistically significant reduction in mean blood CD4⁺ T cells to 33% of pre-drug levels (p=0.0024). CD4⁺ T cells returned to normal levels within 5 days after each treatment period. CD8⁺ T cells were not affected by FTY720 treatment. None of the 3 untreated macaques exhibited significant reductions in CD4⁺ or CD8⁺ T cells. SHIV_{SF162P2} viremia typically peaks within 3 weeks of initial infection and returns to low virus set-points within 12 weeks. FTY720 treatment did not lead to significant deviations from this pattern of viral control. Plasma viral loads progressed from a range of 10⁴-10⁷ copies/ml before treatment to 10⁴-temporarily undetectable levels on the last day of treatment. SHIV_{SF162P2} was not eliminated, however, as plasma viremia and proviral DNA persisted during the 47 days of follow-up.

Conclusions

FTY720 administration did not result in a significant decrease or increase in SHIV_{SF162P2} burden in these macaques, indicating no therapeutic effect at the doses and schedules outlined here. This study has identified an effective FTY720 regimen that reduces blood CD4⁺ cells in rhesus macaques. Future work is needed to evaluate whether this regimen can also reduce rectal or vaginal mucosal CD4⁺ cells and thereby decrease susceptibility to SHIV infection in a preclinical macaque model.

HYPOTHESIS

Altered lymphocyte trafficking during chronic SHIV infection could result in a decrease in viral load due to changes in the activity of CD8⁺ T cells.

APPROACH

Alterations in lymphocyte trafficking were induced with the drug FTY720. 3 SHIV_{SF162P2}-infected rhesus macaques were treated with FTY720. Changes in viremia, lymphocyte presence in peripheral blood, and the activity of CD8⁺ T cells were determined.

Figure 1: Experimental Design

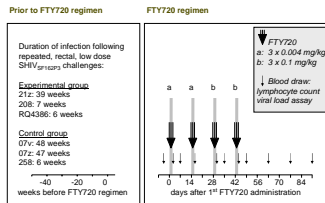


Figure 1: Experimental Design

6 SHIV_{SF162P2}-infected, male Rhesus macaques were enrolled into the study. FTY720 is expected to alter lymphocyte trafficking behavior in peripheral blood in a dose-dependent way. An effective dose for FTY720 has not been reported for Rhesus macaques. FTY720 was therefore given at the low dose of 0.004 mg/kg, administered intravenously on 3 subsequent days as indicated by the triple arrows. The regimen was repeated 14 days later. The drug dose was subsequently increased to 0.1 mg/kg on days 28, 29, 30, 42, 43 and 44. Effectiveness of FTY720 in determining lymphocyte trafficking behavior was evaluated by enumerating lymphocytes from peripheral blood collected at the indicated time points (single arrows). Virus load in plasma and activation markers of CD8⁺ T cells were also evaluated. All animal procedures were approved by CDC's Institutional Animal Care and Use Committee. Previous infection history of the study macaques (left panel): 5 macaques (21z, 208, RQ4386, 07z, and 258) became infected during once weekly, rectal SHIV_{SF162P2} challenges at 10 TCID₅₀ per exposure. These macaques had served as controls for other studies and did not receive other treatment except for virus exposures. Macaque 07v was previously part of a pre-exposure chemoprophylaxis trial with anti-retroviral drugs for the prevention of infection. 07v became infected with a rectal SHIV_{SF162P2} dose of 1000 TCID₅₀ while receiving daily oral Truvada (22 mg/kg TDF and 20 mg/kg FTY) for 1 week before and 4 weeks after virus challenge. At the time of enrollment into the current study, the macaques had been infected for the indicated times, and 07v had not received any anti-viral drugs for 46 weeks.

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RESULTS 1

Figure 2: Identification of an effective FTY720 dose that leads to a reduction in CD4 T cells from peripheral blood

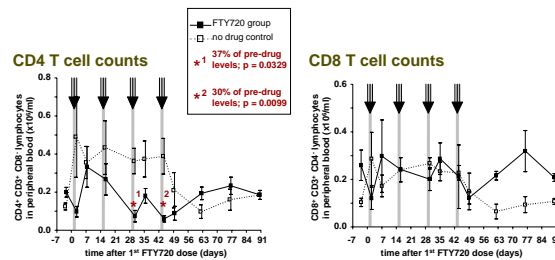


Figure 2: Lymphocyte counts in peripheral blood following FTY720 administration. Only the 0.1 mg/kg FTY720 dose resulted in a statistically significant reduction in mean blood CD4⁺ T cells to 37% of pre-drug levels (day 30, p=0.0329) or 30% (day 45, p=0.0099). CD4⁺ T cells returned to normal levels within 5 days after each treatment period. CD8⁺ T cells were not affected in a measurable way by FTY720 treatment. Although the 3 untreated macaques exhibited fluctuations in T cell counts during the study, none showed significant reductions in CD4⁺ or CD8⁺ T cells compared to the pre-treatment time point during the study. CD4⁺ and CD8⁺ T cells were enumerated by flow cytometry using antibodies to CD3, CD4, CD8 and CD19 (data not shown). Statistical significance was determined by unpaired student's t-test of mean data and the standard error of the mean.

RESULTS 2

Figure 3: FTY720 administration to 3 macaques infected with SHIV_{SF162P2} does not result in uniform reductions in plasma viral load.

Viral Load prior to and during FTY720 regimen

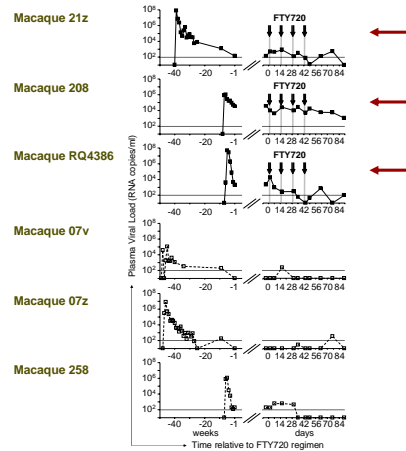


Figure 3: Viral load prior to (left panels) and during FTY720 regimen (right panels). FTY720 administration to 3 macaques infected with SHIV_{SF162P2} did not result in uniform reductions in plasma viral load. SHIV_{SF162P2} viremia typically peaks within 3 weeks of initial infection and returns to low virus set-points within 12 weeks. FTY720 treatment did not lead to significant deviations from this pattern of viral control. Plasma viral loads progressed from a range of 10⁴ copies/ml (monkey 208), 10³ copies/ml (RQ4386), and 10² copies/ml (21z) before treatment to 10⁴-temporarily undetectable levels on the last day of treatment. SHIV_{SF162P2} was not eliminated, however, as plasma viremia and proviral DNA persisted (not shown) during the 47 days of follow-up. The plasma viral load (RNA copies/ml) was determined by PCR using an in-house assay with a detection limit of 100 viral RNA copies per milliliter (Subbarao S, Otten RA, Ramos A et al., J. Infect Diseases 2006, 194: 904-911).

RESULTS 3

Figure 4: The activity of CD8⁺ T cells does not increase during or following FTY720 treatment of SHIV infected macaques.

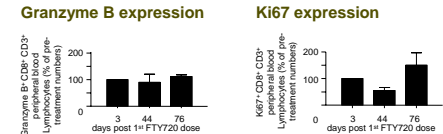


Figure 4: The activity of CD8⁺ T cells does not increase during or following FTY720 treatment of SHIV infected macaques. To determine changes in the function of cytotoxic T cells, markers of cytotoxicity (granzyme B) or of recent proliferation (Ki67) were analyzed. Granzyme B- or Ki67-expressing CD8⁺ CD8⁻ cells were enumerated by flow cytometry using peripheral blood from the 3 FTY720-treated macaques at the indicated time points. Although there was a transient drop in Ki67-expressing CD8⁺ CD8⁻ cells to 55% of pre-treatment levels (p=0.0198, student's t-test) on day 44 of the regimen, there was no sustained change with statistical significance in either of the two markers following the FTY720 regimen. In addition, there were no statistical differences between the treated and untreated groups on days 16, 44 or 76 (data not shown) during or following FTY720 treatment.

RESULTS 4

Figure 5: FTY720 affects the trafficking behavior of CCR5⁺ CD4⁺ cells.

Rationale: Future prophylactic uses of FTY720 could be envisioned. Lymphocyte trafficking drugs could potentially be used to decrease the number of CCR5⁺ CD4⁺ cells at mucosal surfaces. This could reduce susceptibility to HIV-transmission, if CCR5⁺ cells could become unavailable for infection due to altered location. We therefore determined whether CCR5⁺ CD4⁺ cells are sensitive to FTY720 treatment.

Effect of FTY720 on CD4⁺ CCR5⁺ cells

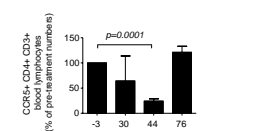


Figure 5: FTY720 affects the trafficking behavior of CCR5⁺ CD4⁺ cells. To determine whether CCR5⁺ CD4⁺ cells are sensitive to FTY720 treatment, we enumerated the cells in peripheral blood. Compared to pre-treatment levels, CCR5⁺ CD4⁺ CD8⁻ lymphocytes from peripheral blood dropped to 64% and to 24% on days 30 and 44 of the FTY720 regimen. This was only statistically significant for the day 44 time point (p=0.0001, student's t-test). For this time point only, there was also a statistical difference between the treated and untreated groups (p=0.0159, student's t-test; data not shown). The drop in CCR5⁺ CD4⁺ cells was not sustained long-term after FTY720 treatment, and cell numbers were similar to pre-treatment levels by day 76. CCR5⁺ CD4⁺ CD8⁻ cells were enumerated by flow cytometry on days -3, 30, 44 and 76 of the regimen.

CONCLUSIONS

- Rhesus macaques respond to FTY720
- A regimen was identified that leads to the transient reduction of CD4⁺ T cells in peripheral blood of SHIV-infected rhesus macaques (regimen: 0.1 mg/kg on 3 consecutive days, given intravenously).
- Therapeutic FTY720 administration did not result in a decrease or increase in SHIV_{SF162P2} plasma viremia at the doses and schedules outlined here.
- No changes in cytotoxic T cell activity were noted.
- Peripheral CD4⁺ CCR5⁺ cells are sensitive to FTY720 administration. FTY720 may have the potential to reduce infection target cells at mucosal surfaces and thus reduce virus transmission potential. Any prophylactic benefit from FTY720 remains to be determined.