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Preclinical Evaluation of the Immunomodulatory Lymphocyte Trafficking Drug FTY720 for HIV Prevention in the Female Genital Mucosa of Macaques

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Abstract

FTY720 has been shown to reduce inflammatory cytokines and immune cells in the genital mucosa of macaques. This pilot study examined the ability of FTY720 to inhibit HIV acquisition. Systemic treatment with FTY720 failed to prevent or delay vaginal SHIV transmission.

Introduction

In the absence of an efficacious vaccine, microbicides and systemic pre-exposure prophylaxis (PrEP) provide important tools for HIV prevention. To date, non-antiretroviral therapy (ART) based microbicides have failed to show efficacy, or have demonstrated toxic and pro-inflammatory effects after repeated application [2, 4]. Inflammation plays an important role in HIV transmission [5], thus dampening innate immunity and target cell recruitment may be a complementary strategy to ART-based microbicides. One immunomodulatory compound, glycerol monolaurate prevented vaginal SHIV transmission in a macaque model [7]. Testing of other potential microbicide candidates in macaques may identify new pathways for HIV prevention.

FTY720 (Fingolimod) is an immunomodulatory agent which alters lymphocyte migration by binding to sphingosine-1-phosphate receptors on lymphocytes and results in their sequestration in secondary lymphoid organs [9]. FTY720 has been approved for human use to treat relapsing multiple sclerosis (MS) [1]. It has also limited viral dissemination of HIV in a humanized mouse model [10]. Previous studies in pigtail macaques indicated that systemic and topical administration of FTY720 reduced levels of cervovaginal inflammatory

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cytokines, and decreased inflammatory scores in histological examination of vaginal tissue [15]. In this pilot study, we examined whether systemic administration of FTY720 could alter SHIV susceptibility in pigtail macaques during intravaginal repeat low-dose challenge.

Materials and Methods

Macaques

Female pigtail (*Macaca nemestrina*) and cynomolgus macaques (*Macaca fascicularis*) were housed by the Centers for Disease Control and Prevention (CDC) according to the Guide for the Care and Use of Laboratory Animals [11]. The CDC Institutional Animal Care and Use Committee (IACUC) approved all described procedures.

FTY720 administration and Virus Challenge

Pigtail macaques were treated with either intravenous (IV) FTY720 (n=3; Pki2, Pdt1, Pvi2), or saline (n=3; Poh2, 96P025, 96P058). FTY720 (Cayman Chemical, Ann Arbor MI) was dissolved in 0.85% physiological saline, and administered weekly at a dose of 0.1mg/kg [6], followed by virus challenge 48h post-treatment (Fig1a). SHIV_{SF162P3} (NIH AIDS Research and Reference Reagent Program) was administered using an established model of repeat low dose vaginal exposures at 50 TCID₅₀ [13]. Viral load was measured by PCR as previously described (detection limit 50 copies/ml) [14]. We employed a log-rank test to compare number of exposures required to infect, and Mann-Whitney to compare peak viremia using GraphPad Prism (La Jolla, CA).

Lymphocyte Analysis

Lymphocytes were isolated from the vagina and cervix of four SHIV+ cynomolgus macaques at necropsy as previously described [3]. All macaques were approximately late luteal stage, and two received IV FTY720 48 hours prior to necropsy. Briefly, intact tissues were cut into fine pieces and treated with multiple rounds of digestion with collagenase, hyaluronidase and elastase at 37°C. Mononuclear cells were purified by Lymphocyte Separation Medium (MP Biomedical, Santa Ana, CA) and frozen in 10% DMSO/ 90% Fetal Bovine Serum. Total cell recovery was determined by an automated Guava cell counter (Millipore, Billerica MA) and was adjusted by the weight (g) of the tissue prior to digestion. All antibodies were from BD Biosciences (San Jose, CA) except CD45 (Miltenyi Biotech, Auburn CA), and fixable live/dead dye (Invitrogen, Grand Island NY). Samples were acquired on a LSRII flow cytometer (BD Biosciences) and analyzed using FlowJo (Treestar, Ashland OR). Due to the terminal nature of these experiments, we were limited in the use of cynomolgus macaques for a descriptive study without statistical analysis.

Results

Six female pigtail macaques were treated with either FTY720 (0.1mg/kg) or saline for two weeks; then weekly treatment was followed by a repeat low-dose SHIV_{SF162P3} vaginal challenge 48 hours post treatment (Fig1A). SHIV susceptibility was not altered in the FTY720 treated macaques compared to control animals, as all treated macaques and controls were infected after a median of four and seven exposures, respectively (p>0.05) (Fig 1B).

Despite continued FTY720 treatment, peak viremia did not differ from untreated macaques ($p>0.05$) (Fig 1C). No adverse clinical events were reported in the FTY720 or saline-treated animals.

To further examine possible changes to cell populations in the reproductive tract by FTY720, and to understand its failure to prevent SHIV infection, we necropsied the female reproductive tract of four SHIV+ cynomolgus macaques, two receiving systemic FTY720 48 hours prior to necropsy. Similar to the pigtail macaques, treatment with FTY720 reduced the number of cells recovered from the blood (Fig 2A). However, the total number of cells recovered from the necropsied vaginal and cervical tissue, normalized by tissue weight, was not significantly different (Fig 2B). To eliminate possible contaminating epithelial cells in the tissues isolations, we gated on CD45 to identify leukocytes and measured the percent of CD3+ T cells. There was a reduced percent of T cells in the blood, but not in the cervical or vaginal tissue of the FTY720-treated macaques (Fig 2C).

Discussion

This study evaluated the lymphocyte trafficking drug FTY720 as an immunomodulator of lymphocytes trafficking to the vaginal mucosa. Despite our previous studies reporting reduced genital inflammatory indicators[15], the drug regimen was not efficacious in preventing or delaying vaginal SHIV infection.

Previous histological studies demonstrated reduced infiltrating lymphocyte populations in the epithelial layers of vaginal tissue post FTY720 treatment[15]. However, when we examined the total tissue, including the epithelial and submucosa, and we observed no changes in CD3+ T cell numbers. Notably, this method would be unable to reveal changes in immunological microenvironments along the vaginal canal, did not distinguish between the ecto- and endocervix [8, 12], and did not look specifically at CD4 target cells in these SHIV infected animals. It has not been resolved whether vaginal, cervical, or other reproductive tissues are the primary route of viral entry. In addition, our study does not rule out that more frequent dosing, such as an equivalent to human use for MS, an oral dose of 0.5mg/day[1], may result in changes to the resident immune populations of the reproductive tract.

One study limitation was the small number of animals. Studies that would be appropriately powered and with modified drug dosing were not pursued following the outcome of this pilot study. This study signals a need to further assess whether genital immune manipulation is a viable strategy for HIV prevention.

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(A) Study design

(B) Kaplan-Meier Survival Curve

(C) Plasma SHIV_{SF162P3} RNA levels **Figure 1. Study design and SHIV_{SF162P3} infection data**

Study Design: (A) Macaques were treated weekly with 0.1mg/kg IV FTY720 or 0.85% physiological saline. After two initial weeks, 48 hours post each treatment the animals were challenged with a vaginal repeat low-dose of SHIV_{SF162P3}. Virus challenges were stopped once an animal was confirmed infected, but FTY720 or saline was continued once weekly for 6 weeks. (B) Kaplan-Meier survival curve of macaques remaining uninfected after the indicated number of repeated vaginal virus challenges. A Log-rank test was used to compare the number of exposures to infection between groups. (C) SHIV_{SF162P3} viral load kinetics for each saline or FTY720-treated macaque.

Figure 2. Cell recovery in the FRT 48h post FTY720 treatment

Cells were isolated from the blood and female reproductive tract (FRT) of two untreated cynomolgus macaques and two macaques treated with IV FTY720. Total number of isolated cells enumerated by a Guava automated cell counter in the (A) blood; and (B) tissues. To account for size, total cell number was divided by the weight of the starting tissue (in grams). (C) Flow cytometry analysis of CD3+ T cell percentages of total leukocytes (CD45+) in the blood and tissues following FTY720 treatment. CD45+ cells were gated from total cells and dead cells were eliminated by live/dead staining. The T cell frequency of

one vaginal sample was not included due to low viability. Open symbols: untreated (untx), closed symbols: FTY720-treated animals.