

Prevention of Human Immunodeficiency Virus Type 1 Transmission by Pharmaceuticals Targeted to Host Proteins Required for Virus Infection? Consideration of Farnesyl Thiosalicylic Acid, a Ras Inhibitor

A Robert Neurath^{1*}, Carol Lackman-Smith²

¹Virotech, 1496 Hemlock Farms, Hawley, PA 18428, USA

²Southern Research Institute, 431 Aviation Way, Frederick, Maryland 21701, USA

Abstract

Recent success in defining the human immunodeficiency virus type 1 (HIV-1) – host cell protein interaction network has provided an opportunity for development of novel antiviral therapeutics targeted to host proteins required for virus infection. This expanded earlier successful development of antagonists for the cellular receptors (CD4) and co-receptors (CCR5 or CXCR4) involved in virus attachment. Induction of the G- α_q signaling cascade by the HIV-1 envelope is required for virus entry, and it's blocking prevented HIV-1-mediated membrane fusion and initiation of infection. One of the blockers, the Ras inhibitor *S-trans, trans*-farnesylthiosalicylic acid (FTS), was reported to interfere with HIV-1 infection. Since FTS appears to have an established safety record and is being evaluated (as *Salirasib*, oral) in phase II human clinical trials for treatment of lung cancer, it was of interest to evaluate the potential of FTS as a topical microbicide for prevention of sexual transmission of HIV-1. Data shown here indicated that this compound did not meet the criteria of an established screening algorithm for evaluation of topical microbicides. Nevertheless, the possibility remains to be explored that FTS (especially when used in combination with other anti-HIV drugs) might be useful in sustained pre-exposure prophylaxis to prevent HIV-1 transmission.

Keywords: Human immunodeficiency virus type 1; HIV-1; Microbicides; Sexual transmission; Prophylaxis; Farnesyl thiosalicylic acid; CCR5; CXCR4; TAK 779. AMD 3100

Abbreviations: FTS: *S-trans, trans*-Farnesyl Thiosalicylic Acid or Farnesyl Thiosalicylic Acid; PBS: Phosphate Buffered Saline; DMSO: Dimethylsulfoxide; CCR5 and CXCR4: Coreceptors for R5 and X4 tropic HIV-1; IC₅₀ (IC₉₀): Inhibitor Concentrations at which virus replication is reduced to 50% (90%) of that observed in the absence of inhibitor; TC₅₀: concentration of a compound at which 50% of cells, in the absence of virus, remain viable.

Introduction

The global AIDS epidemic has proceeded without abatement for about 30 years with about 36 million people having chronic HIV-1 infections and about 36 million who already succumbed to AIDS. Most new infections have been acquired by the mucosal route, heterosexual transmission playing the major (~80%) role. Anti-HIV-1 vaccines applicable to global immunization programs are not expected to become available for many years. Therefore, other prevention strategies are needed, i.e. mechanical or chemical barrier methods. The latter correspond to microbicides, topical formulations expected to block HIV-1 infection (and possibly also transmission of other sexually trans-

mitted pathogens) when applied vaginally or rectally before intercourse. Several large-scale phase III efficacy trials of candidate microbicide formulations failed to demonstrate efficacy. Therefore, additional research and development in the microbicide field is needed (Balzarini and Van Damme, 2007; Cutler and Justman, 2008; Hendrix et al., 2009). Formulations of several anti-retroviral drugs targeted to HIV-1 proteins or to cell receptors (CD4) or co-receptors (CCR5) for the virus have been considered for this purpose (Schols, 2004; Moore et al., 2004; Vermeire et al., 2006). Since HIV-1 with tropism for CCR5 (= R5 viruses) are preferentially transmitted by the mucosal route, CCR5 antagonists have been primarily considered for development of microbicides.

Application of anti-HIV-1 pharmaceuticals targeted to HIV-1 proteins may lead to the emergence of drug-resistant virus variants. Recent success in defining the HIV-1—host cell interaction network has provided an opportunity for development of novel antiviral therapeutics targeted to host proteins (in addition to CD4, CCR5 and CXCR4) required for virus infection (Kellam, 2006; Zhang et al., 2007; Nguyen et al., 2007; König et al., 2008; Ptak et al., 2008; Zhou et al., 2008; Loo and Gale, 2008; Goff, 2008; Brass et al., 2008; Bushman et al., 2009; Fu et al., 2009). The promise of this new approach has been supported by the finding that inhibitors of the G- α_q signaling cascade, required for HIV-1 entry into cells, interfere with virus-mediated membrane fusion and infection (Harmon and Ratner, 2008). Among the thirteen identified active compounds, targeted to distinct host

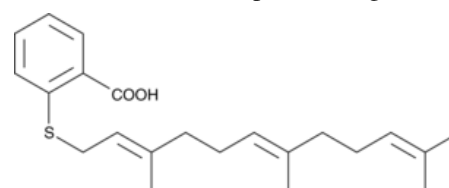


Figure 1: *S-trans, trans*-Farnesyl Thiosalicylic Acid (FTS; *Salirasib*). CAS Number 162520-00-5.

*Corresponding author: A Robert Neurath, Virotech, 1496 Hemlock Farms, Hawley, PA 18428, USA, Tel: (570) 775 7877 or (212) 529 4584; E-mail: rneurath@att.net

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proteins, *S-trans*, *trans*-farnesyl thiosalicylic acid (Figure 1; FTS) has been in human phase I and II clinical trials (Tsimberidou et al., 2009; Bothakur et al., 2007; <http://clinicaltrials.gov/ct2/show/NCT00531401>). FTS is a Ras farnesylcysteine mimetic which disrupts the association of active Ras proteins with cell membranes and membrane microdomains, and is being considered for chemotherapy of solid tumors (Halaschek-Wiener et al., 2003; Goldberg et al., 2008; Rotblat et al., 2008).

The already ongoing human clinical trials of FTS (oral Salirasib) make this compound an attractive candidate for possible development as a topical microbicide to prevent HIV-1 transmission. As a first step in this consideration, the inhibitory activity of FTS against infection of cells *in vitro* was evaluated by a primary assay of a screening algorithm for discovery of topical microbicides (Lackman-Smith et al., 2008). The R5 virus, HIV-1 BaL, was selected for the tests since R5 HIV-1 strains are preferentially transmitted by the mucosal route (Moore et al., 2004).

Materials and Methods

Cells and virus

MAGI-CCR5 cells, expressing the HIV-1 receptor CD4 and co-receptor CCR5 and beta-galactosidase under the control of the HIV-1 long terminal repeat (LTR), and HIV-1 BaL were obtained from the National Institutes of Health (NIH) AIDS Research and Reference Program (Germantown, MD 20874).

Pharmaceuticals

The CXCR4 and CCR5 antagonists AMD3100 and TAK-779, respectively, were obtained from the NIH AIDS Research and Reference Program, and handled as described (Lackman-Smith et al., 2008). *S-trans*, *trans*-Farnesyl-Thiosalicylic Acid was obtained from Cayman Chemical Company, 1180 E. Ellsworth Road, Ann Arbor, MI 48108.

Data analysis and quality control

For each assay, IC_{50} , IC_{90} and TC_{50} values were calculated using regression analysis. The Therapeutic Index (TI) was calculated as TC_{50}/IC_{50} . All assays passed internal quality standards established at the Sothorn Research Institute, Frederick, MD 21701. This included quality of replicates, endpoint signal and performance of assay controls.

Solutions of the latter compound were prepared as follows:

1. FTS was dissolved in 100% dimethylsulfoxide (DMSO) to a stock concentration of 40mM by adding 0.2022 mL DMSO to 2.9 mg FTS.
2. Another stock solution was prepared by first dissolving 0.7 mg FTS in ethanol and then adding an equal volume of phosphate buffered saline (PBS) to achieve a final concentration of 0.5 mg/mL (=1.4 mM).
3. FTS was fully soluble using both methods.
4. For the assays, each stock solution was used to prepare 2x concentrated working solutions and serial dilutions were prepared starting from a final concentration of 100 μ M. The diluents, DMSO and ethanol- PBS (1:1) were also tested as controls in the assays at the same concentration as that used for the test material. Log_{10} dilutions of all working solutions of test article or diluent control were prepared for testing.

Assays for inhibition of HIV-1 infection

The assays were performed as described earlier (Lackman-Smith et al., 2008). Briefly, 24 hours prior to initiation of the assay, the cells were trypsinized, counted, and plated in 96-well flat bottom wells at 1×10^4 cells per well. Medium was removed and diluted test article or diluent controls in medium placed on the cells, and incubated for 15 min at 37°C. Ten (10) TCID₅₀ of the BaL strain of HIV-1 was then added to the wells and the plates were incubated for 40 to 48 h at 37 °C. At termination of the assay, media was removed and β -galactosidase enzyme expression determined by chemiluminescence per the manufacturer's instructions (Tropix Gal-screen β , Applied Biosystems, Bedford, MA 01730). TAK-779 and AMD3100 were the positive and negative control compounds respectively for the assay. Toxicity using CellTiter96® Reagent (Promega, Fitchburg, WI 53711) was tested in parallel with tests for inhibition of HIV-1 infection. All assays were performed in triplicate and mean values were calculated.

Results

To confirm the proper performance of infectivity assays, the inhibitory effect of the CCR5 antagonist TAK 779 on infection

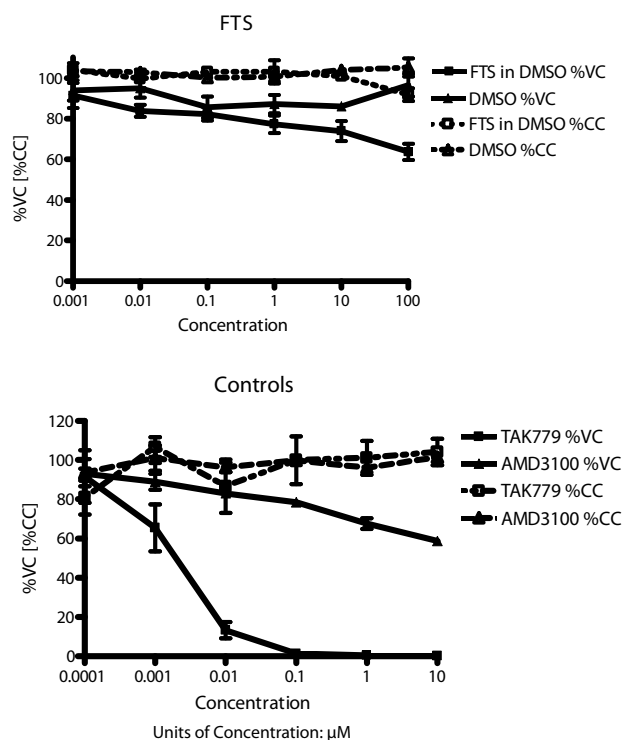


Figure 2: Dose response curves for inhibition of HIV-1 BaL infection (%VC) of cells by FTS (top panel); and by the compounds TAK 779 and AMD 3100, respectively (bottom panel); and for cytotoxicity (%CC) to uninfected cells. The FTS stock solution (40 mM) was prepared in DMSO. The effect of DMSO alone on virus infection and its cytotoxicity were determined at final concentrations corresponding to those of FTS dilutions (top panel). The scales on the ordinates of each panel correspond to both %VC and %CC. MAGI-CCR5 cells, expressing CD4, the HIV-1 co-receptor CCR5, and beta-galactosidase under the control of the HIV-1 long terminal repeat (LTR), were used. HIV-1 BaL uses the CCR5 co-receptor (= R5 tropic), and TAK 779 is a specific CCR5 antagonist while AMD 3100, used as a “negative” control, is a CXCR4 antagonist. Virus infection was monitored by chemiluminometric quantitation of beta-galactosidase (relative quantity corresponding to infection in the absence of any compounds listed above = 100%). Cytotoxicity was determined using the CellTiter Reagent (Promega). Absorbance measured by the assay is directly proportional to the number of living cells in culture (=100%).

of MAGI-CCR5 cells by the R5-tropic HIV-1 BaL was measured. The results are shown in Figure 2 (bottom panel). The IC_{50} and IC_{90} values, calculated using regression analysis, were 2 and 20 nM, respectively. Cytotoxicity was minimal, and the calculated therapeutic index (TI) was >5,000. This conforms to already established results (Lackman-Smith et al., 2008).

The CXCR4 antagonist AMD 3100 had, in comparison, marginal effects, as expected ($IC_{50} = >10 \mu\text{M}$).

FTS was even less inhibitory than AMD 3100 (Figure 2, top panel; $IC_{50} = >100 \mu\text{M}$). These results were obtained using a stock solution of FTS prepared in DMSO. The medium containing DMSO only had no detectable inhibitory effects. Similar results were obtained with an FTS stock solution prepared in an ethanol-PBS mixture (data not shown).

In conclusion, the inhibitory activity of FTS on HIV-1 BaL infection was too low for further consideration of this compound for inclusion into topical microbicide formulations.

Discussion

The inhibition of HIV-1 BaL infection of the MAGI-CCR5 target cells by FTS was unimpressive (~40% inhibition at a 100 μM concentration) but significant in comparison with that attributable only to the diluent containing DMSO (Figure 2, top panel). The observed inhibition could not have been caused by cytotoxic effects of the compound. Nevertheless, these data suggest that FTS does not meet criteria for further development as a topical microbicide. These results merit further discussion, and should not discourage from additional research regarding the potential application of FTS for pre-exposure prophylaxis.

Results of earlier experiments (Harmon and Ratner, 2008) showed that FTS at a 50 μM concentration caused a 99.7 +/- 2 % inhibition of infection. The R5 virus HIV_{YU2} and TZM-BL cells were used in these experiments. More significantly, the cells were pre-incubated with FTS for 1 hr prior to the addition of virus, as compared with 15 min in the experiments described in the Results section. An appropriate duration of pre-exposure to a compound targeted to host cell proteins before exposure to HIV-1 is likely to be required for optimal expression of antiviral activity. In this respect it should also be mentioned that inhibition of Ras activation by FTS and other compounds was performed with serum-starved cells (Harmon and Ratner, 2008). This was not so when inhibition of HIV-1 infection was measured. Pre-exposure to compound with anti-HIV-1 activity for 1 hr or longer is impractical for topical microbicides to be applied vaginally or rectally before intercourse.

The limited success of microbicide efficacy trials has led to the consideration of oral pre-exposure prophylaxis with anti-retroviral drugs to prevent sexual transmission of HIV (Garcia-Lerma et al., 2008; Cohen et al., 2008). In the latter setting, pharmaceuticals targeted to host cell proteins essential for HIV-1 replication (including FTS) may find advantageous new applications. Methods for sustained continuous release (e.g. from a vaginal ring) should be considered in this regard. Continual topical FTS release would likely overcome problems due to the relatively short half-life of orally administered FTS (about 3hr; Tsimberidou et al., 2009) and the dose-dependent potential side effects (diarrhea; completely correctable with oral antidiarrheals) of the compound applied systemically (Borthakur et al., 2007).

Animal model experiments will be required in order to investigate further the potential of FTS for anti-HIV-1 prophylaxis.

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