Treating Post-HIV Infection Through Molecular Target of HIV TAT and PKC Regulation with Berberine and Curcumin

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Translational Medicine

Rationale: HIV TAT protein is a crucial factor that promotes viral transcription. Its capability to agonize PKC is vital to HIV pathogenesis as it consequently allows the recruitment of P-TEFb complex, ultimately causing the viral transcription process to surpass the proximal pausing site at RNA Polymerase II and leading to faster virus replication. With a lack of diversity in the mechanisms for the development in current post-HIV infection drugs, it is critical to find a novel route that may be able to both treat the infection at the same time of avoiding potential adverse events that are associated with existing treatments. Berberine and Curcumin will beused in this study to counter the harm that can be induced by TAT protein. Berberine is currently in usage for several intestinal inflammatory illnesses, and its ability to inhibit PKC serves the essential purpose in this study as TAT protein functions by activating it. Curcumin, on the other hand, causes proteasomal degradation of TAT protein, reducing the viable TAT protein concentration and downshifting the probability of TAT-PKC interaction. Since both herbal-derived chemicals have high, flexible dosage allowance and are suggested to have no obvious side-effects, Berberine and Curcumin might be valid candidates in treating HIV infection.

Hypothesis: Since Berberine and Curcumin are able to individually hinder the pathogenic function of TAT protein through distinct mechanisms, applying a dual-treatment with both chemicals may be more effective than utilizing one only.

Material and Methods:

1). Cell line: NIH-3T3 fibroblasts. Source: Mus musculus, mouse, embryo. It will be obtained from American Type Culture Collection (ATCC).

2). Cell Growth and Maintenance: The fibroblast NIH-3T3 cell line will be grown *in vitro* in MEM media supplemented with 10% fetal bovine serum. Cells will be cultured in a 37°C incubator (NAPCO) supplemented with 95% O_2/5% CO_2.

3). Cell Treatment: TAT protein, Curcumin, Berberine, TPA, and Dimethyl Sulfoxide (DMSO) will be purchased from Sigma-Aldrich; TAT protein will be diluted using only distilled water while all of the rest will be dissolved in DMSO prior to being diluted with 50% H_2O/50% DMSO solution. Expected concentration of treatment dilutions will be the following: TAT (100x = $3.21*10^{-1}$ uM), Berberine (100x = $2.98 *10^{-2}$ uM), Curcumin (100x = $2.72 * 10^{-2}$ uM), and TPA (100x = 8.56 uM). Trypsin will be applied during cell seeding to detach all the 3T3 cells from the culture flasks prior to entering the centrifuge process, which is crucial to separate cell pellets from the supernatant. A total of six treatment groups will be used in cell migration assay: Control, TAT alone, Curcumin + TAT, Berberine + TAT, Berberine + Curcumin +TAT, Berberine + Curcumin. The same groups will also be applied to Enzyme-Linked Immunosorbent Assay and MTT Assay. For MTT Assay, additional groups of TPA alone, TPA + Berberine, TPA + Curcumin, and TPA +Curcumin + Berberine will be used as well.

3). Assays to assess TAT protein's impact *in vitro*: Cell Migration Assay, MTT Cell Proliferation Assay, and Enzyme-Linked Immunosorbent Assay (ELISA).

- **Cell Migration Assay:** Matrix Metallopeptidase 9 (MMP9), a downstream protein to PKC pathway, can induce cell migration under overexpression. Thus, this assay will assess the TAT protein activity on agonizing PKC. In this assay, a 6-well plate will be used with different treatments applied to each well. Afterwards, the plate is going to be incubated for either 24 or 48 hours for cell migration to take place on the migration bridge created. The migration bridges are straight carvings across the cell colony in the wells using the tip point of a 200 uL pipette tip. After incubation, the wells will be dyed in a process involving Hema 3 fixatives and dye prior to ultimately being examined under light microscope (40x). The length of migration bridges (in pixels) indicates the degree of cell migration.

- **MTT Cell Proliferation Assay**: Using this assay, the TAT protein's function can be examined indirectly as high level of cell inviability is related to high level of PKC agonization. In this assay, a 96-well plate will be used with different treatments. A small molecule 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) will be used; this molecule is able to be coupled with NADH electron receptors in mitochondria, forming purple formazons that can be assessed to determine cell viability. DMSO will also be applied to the 96-well plate after an incubation duration of 90 minutes after prior treatments to both break up the plasma and mitochondrial membrane and dissolve the released formazon, facilitating the ultimate MPM6 (595nm wavelength) analysis to determine cell viability by examining the concentration of purple MTT formazans.
- **ELISA**: 2mL of 3T3 cells will be first applied to each well of a six-well plate; 20µL of each treatment (TAT alone, Curcumin+TAT, Berberine+TAT, Berberine+Curcumin+TAT, and Berberine+Curcumin) will be introduced to five individual wells. The plate will then be incubated for 24 hours and afterwards immediately stored at -20°C for another 24 hours to initiate cold-stress induced cell apoptosis, releasing MMP-9. Then, the cell media will be defrosted and brought to room temperature along with all the reagents and materials provided by the PicoKine MMP-9 ELISA kit. In each trial, 100µL of standard (solution provided for final comparison to determine the concentration of MMP-9; n=16) and sample (n=8) from the cell media will be introduced into each of the individual wells. 100µL sample diluent buffer will be added to each of the wells before the plate is incubated for 90 minutes at 37°C. Afterwards, any liquid from each of the wells will be removed, and 100µL Biotinylated Anti-Human MMP-9 detection antibody provided by the kit will be added to each of the wells. The plate is then going to be incubated for another 90 minutes at 37°C and washed with phosphate-buffered saline for three times before the removal of remaining liquid in each of the wells. 100µL of the Avidin-Biotin-Peroxidase Complex (ABPC) will then be introduced in each well to bound with MMP-9, and the plate is going to be incubated for another 30 minutes at 37°C. The unbounded ABPCs are then to be washed away with wash buffer, and the wells will be dried again. At this point, the plate will be incubated

for 20 minutes at 37°C after 90μL of Color Developing Reagent is introduced into each of the wells. ultimately, 100μL of stop solution is going to be introduced to each of the wells, and its color should immediately turn into yellow; microplate readers will then be employed to read the absorbance, which positively correlates with the MMP-9 concentration, at 450nm.

4). Data Analysis: Student t-test, along with an ANOVA Tukey's post hoc test, will be applied to the analysis. The percentage differences in the outcomes of cell migration assay, cell viability assay, and ELISA will be assessed to determine the efficacy of single and dual-treatment of Berberine and Curcumin.

Goal: This study will be focusing extensively on the degree to which Berberine and Curcumin can deteriorate the function of TAT *in vitro*.

Hypothesis: The dual-treatment with both Curcumin and Berberine will have better efficacy in alleviating TAT protein stimulated PKC upregulation than the treatment of either one individual chemical *in vitro*.

Risk and Safety: gloves and goggles will be applied at all times. Cell culture hood will be used during cell treatment.

1) Trypsin: conducts with this chemical will be under close mentor supervision. According to Thermo-Fisher MSDS, this chemical causes serious eye and skin irritation and respiratory organ toxicity.
2) DMSO: conducts with this chemical will be under close mentor supervision. According to Thermo-Fisher MSDS, this chemical causes potential respiratory injuries upon careless consumption, and it is highly inflammable.
3) TAT protein: this chemical is relatively safe as it cannot cause harm if not entering the blood serum.

4) TPA: this chemical is relatively safe as it cannot cause harm unless through direct exposure to skin or consumption.
5) Curcumin: this chemical causes skin irritation and serious eye irritation through direct contact with the mentioned body parts. It may cause potential respiratory irritation if carelessly breathed into the body..
6) Berberine: Upon careless consumption through breathing, this chemical may be irritating to the mucous membranes and upper respiratory tract.
7) MTT: this chemical causes skin irritation, serious eye irritation, and respiratory irritation upon contact or consumption. At large concentration of intake consumption, this chemical is suspected to cause genetic defects.

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