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UCLA1 aptamer inhibition of human immunodeficiency virus type 1 subtype C primary isolates in macrophages and selection of resistance

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ABSTRACT

We have previously shown that the aptamer, UCLA1, is able to inhibit HIV-1 replication in peripheral blood mononuclear cells (PBMCs) by binding to residues in gp120. In this study we examined whether UCLA1 was effective against HIV-1 subtype C isolates in monocyte-derived macrophages (MDMs). Of 4 macrophage-tropic isolates tested, 3 were inhibited by UCLA1 in the low nanomolar range ($IC_{80} < 29$ nM). One isolate that showed reduced susceptibility (< 50 nM) to UCLA1 contained mutations in the α5 helix next to the CD4 and co-receptor (CoR) binding complex. To further evaluate aptamer resistance, two primary viruses were subjected to increasing concentrations of UCLA1 over a period of 84 days in PBMCs. One isolate showed a 7-fold increase in IC_{80} (351 nM) associated with genetic changes, some of which were previously implicated in resistance. This included F223Y in the C2 region and P369L within the CD4 and CoR binding complex. A second isolate showed a 3-fold increase in IC_{80} (118 nM) but failed to show any genetic changes. Collectively, these data show that UCLA1 can efficiently block HIV-1 infection in MDMs and PBMCs with escape mutations arising in some isolates after prolonged exposure to the aptamer. This supports the further development of the UCLA1 aptamer as a HIV-1 entry inhibitor.

1. Introduction

The AIDS epidemic is a major global health crisis with over 36 million people living with HIV and high rates of new infections in many countries (www.who.int/hiv/en). HIV-1 subtype C is the most dominant viral subtype found mostly in sub-Saharan Africa and India with over 70% of new global HIV infections occurring in these regions. While the development of a vaccine is considered essential to control the HIV epidemic, this has proven to be a major challenge largely due to the high genetic variability and evolutionary rates of HIV [1]. The widespread use of anti-retroviral therapy has had a significant impact on the epidemic with over 15 million people currently being treated. However, anti-retroviral drugs trigger viral evolution and select for resistance [2,3] and as such newer drugs and agents need to be developed.

UCLA1 is a shortened and modified RNA aptamer derived from the parental B40 aptamer [4,5]. This molecule has been shown to bind to HIV-1 gp120 with high specificity and affinity and to potently inhibit HIV-1 subtype C entry. Our previous study revealed UCLA1 binding sites within the CoR binding site, at the base of the V3 loop, and in the bridging sheet within the conserved V1/V2 stem-loop of gp120 [6]. As a promising anti-HIV-1 candidate, UCLA1 may complement existing ARV regimens or be used in HIV prevention strategies, such as a microbicide. The study of resistance against any inhibitor of HIV-1 is necessary to identify the nature and number of mutations associated with viral escape. It also enables studies to assess the prevalence of naturally-occurring resistant strains and hence the likelihood of efficacy.

In addition to $CD4^+$ T lymphocytes, HIV-1 infects blood monocytes and tissue macrophages which are thought to play an important role in the maintenance of viral reservoirs [7,8]. Furthermore, these cells may play a role in HIV-1 transmission at mucosal sites. Thus potential entry inhibitors of macrophage infection could not only block the transmission of HIV at mucosal surfaces but also prevent seeding of reservoirs. Thus, the current study examined UCLA1 inhibition efficacy against HIV-1 subtype C clinical isolates in MDMs and evaluated the generation of UCLA1 escape mutations in PBMCs.

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2. Materials and methods

2.1. Isolation of monocyte-derived macrophages

Macrophages were derived from blood monocytes [9]. Briefly, PBMCs were freshly isolated from HIV-seronegative blood donors using the Ficoll-Hypaque method [10] and re-suspended in Lonza X-VIVO-10 culture media (Whitehead Scientific, S.A), supplemented with 10% fetal bovine serum (FBS, Gibco Invitrogen, South Africa). Cells were incubated for 90 min at 37 °C in 5% CO_2 in T150 flasks and washed to remove non-adherent cells. The adherent monocytes were incubated overnight in the supplemented X-VIVO-10 media and the monolayer disrupted by adding chilled PBS at 4 °C for 1 h [11]. Re-suspended monocytes were cultured in Rosewell Park Memorial Institute (RPMI) medium supplemented with 10% FBS (10% RPMI growth media) and 5 ng/ml human granulocyte macrophage colony stimulating factor (hGM-CSF) (Roche Diagnostics, Mannheim, Germany) for 5–7 days to enable differentiation into macrophages.

2.2. Phenotypic characterization of monocyte-derived macrophages

Phenotype confirmation of the differentiated macrophages was performed by fluorescence activated cell sorting (FACS) analysis [9,11]. Briefly, the macrophages were fixed and indirect cell surface staining performed with CD14/AF514 MAbs (Whitehead Scientific, S.A, Invitrogen Life Technologies, S.A) which stained undifferentiated monocytes, CD68/AF488 or MHCII/AF488 MAbs stained differentiated monocytes (macrophages) and CD3/PE-Cy5.5 (Caltag Laboratories, Thailand) stained T lymphocytes. The cells were acquired on the BD FACSCalibur flow cytometer (Immunocytometry Systems, CA, USA) and analysed with the BD CellQuest Pro software, version 5.1 (BD Biosciences, NJ, USA).

2.3. Screening of macrophage-tropic viruses

A panel of 25 HIV-1 subtype C replication competent primary isolates were screened for macrophage tropism. These included four viruses from the CAPRISA 002 Acute Infection study cohort [12], five from the Durban female sex workers cohort [13], eight paediatric isolates [14], five from HIV/TB co-infected patients and three AIDS patients co-infected with Cryptococcus meningitis [15]. Twenty of the viruses were R5, four were R5 × 4 [14,15] and one X4 virus [15]. An R5-tropic subtype B primary virus, ADA, known to infect macrophages was used as a reference strain. Briefly, viral stocks were grown in 2×10^6 PBMCs which were CD8 T cells depleted using the RosetteSep human CD8 depletion cocktail method (Separation Scientific, South Africa). The cells were re-suspended in RPMI medium plus 20% FBS, 50 μg/ml gentamicin (Sigma-Aldrich, South Africa) and 5% IL-2 (Roche, South Africa) (complete medium). Phytohemagglutinin (PHA) (Sigma-Aldrich, South Africa) was added to the media for activation of the lymphocytes. Culture supernatants were tested weekly for p24 antigen using the Vironostika HIV-1 Antigen Microelisa System (Biomerieux, South Africa) and expanded when high levels of p24 antigen (100–200 ng/ml) were present. Cultures were fed twice weekly with complete medium and weekly with fresh PHA-activated donor PBMCs for 3 weeks. Virus-containing supernatants were clarified by centrifugation and stored at −70 °C until use.

2.4. HIV-1 infection of MDMs

MDMs ($0.5–1.0 \times 10^6$ cells/ml) were infected with 500–2500 $TCID_{50}$ of the primary viruses in 24-well tissue culture plates and incubated overnight at 37 °C, 5% CO_2 [9]. Control wells with only cells and media were included. Virus inoculum was aspirated and the cells washed with 10% RPMI growth media. The cells were then re-suspended in 10% RPMI growth media and incubated for a further 6 days at 37 °C, 5% CO_2. The cultures were harvested every 7th day and maintained for a maximum of 42 days.

2.5. Neutralization of HIV-1 in MDMs

Neutralization of HIV-1 subtype C infection in MDMs by UCLA1 anti-gp120 RNA aptamer was done as previously described for PBMCs [9]. Briefly, UCLA1 was used at a starting concentration of 100 nM with 3-fold serial dilutions in RPMI supplemented with 5% FBS (growth media). Virus supernatants at 500–2500 $TCID_{50}$ were added to the serially diluted aptamer and incubated for 1 h at 37 °C, 5% CO_2. Differentiated macrophages were seeded at 1.0×10^6 cells/ml in 96-well culture plates. Half of the volume of the cell supernatants was aspirated and the aptamer/virus mix added to the cells and incubated overnight at 37 °C, 5% CO_2. The assay was performed in triplicate and included control wells without aptamer. The cells were washed 3 times by aspirating the virus inoculum from each well and adding 250 μl of RPMI growth media. The cells were then incubated for a further 6 days in RPMI growth media at 37 °C, 5% CO_2. On day 7, 50 μl of virus supernatants were harvested from each well and the cultures replenished with an equal volume of RPMI growth media and incubated at 37 °C, 5% CO_2 for a further 7 days. The supernatants were harvested every 7th day for a maximum of 21 days. The harvested viral supernatants were inactivated with 1.25% Empigen BB detergent and stored at 4 °C in zip-lock plastic bags until further use.

2.6. HIV-1 p24-antigen immunoassay

Macrophages were evaluated for infection and neutralization using the HIV-1 chemiluminescent p24 antigen ELISA as previously described [16]. Briefly, p24 antigen was captured from a detergent lysate of virions by a polyclonal antibody adsorbed to 96-well plate. Bound p24 was detected with an alkaline phosphatase-conjugated anti-p24 MAb and a luminescent detection system. Luminescence was measured with the Wallac 1420 Victor Multilabel luminometer (Separation Scientific, S.A). The neutralization titers were determined at 70% level of reduction in p24 antigen production of the test cultures compared with the negative control cultures (without inhibitor).

2.7. Evaluation of resistance to UCLA1

Two primary isolates that showed different co-receptor usage, RP1 (R5 × 4-tropic) and Du422 (R5-tropic) were used to evaluate resistance to the aptamer. Both viruses were shown to be sensitive to UCLA1 in PBMCs in the previous study [6]. For this, 1000 $TCID_{50}$ of each virus was cultured in the presence of increasing concentrations of the aptamer in 1×10^6 CD8-depleted PBMCs. The starting concentration of the aptamer was the IC_{80} of each virus tested. Virus cultures without UCLA1 were used as negative controls. All cultures were maintained in 5% RPMI growth media supplemented with 0.05 μg/ml of IL-2. The cultures were passaged every 7th day and new cultures set up with 10% of the harvest from the previous culture in freshly isolated CD8-depleted PBMCs. UCLA1 concentration was increased whenever the viral growth, measured by p24 antigen ELISA, was similar or higher than the negative control cultures.

2.8. Isolation, amplification and sequencing of HIV-1 RNA

Isolation of viral RNA from cultures was performed with the QIAamp mini spin viral isolation kit (QIAGEN, S.A). The extracted RNA was reverse transcribed into cDNA using OFM19 primer: