Inhibition of HIV cell-to-cell fusion by antiretroviral drugs and neutralizing antibodies

Diniz AR[1], Borrego P[1], Bártolo I[1], Taveira N[1,2]

[1]Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, The University of Lisbon, Lisbon, Portuga; [2] Instituto Superior Ciências da Saúde Egas Moniz, Monte de Caparica, Portugal

Introduction

Inhibition of HIV cell entry by antiretroviral drugs and neutralizing antibodies (NAbs) is typically measured in assays where cell-free virions enter reporter cell lines. However, direct Env-mediated cell-to-cell transmission is a major mechanism of HIV infection that also needs to be targeted. In this work we aimed to determine the ability of anti-HIV compounds in clinical or research use to inhibit HIV mediated cell-to-cell fusion (syncytia formation).

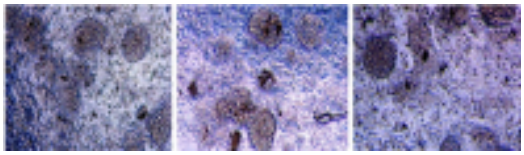

Figure 1 -Microscopic observation of syncytia for untreated Hela and TZM-bl cells. (magnification:100X)

Methods

We developed a new method in which Hela-CD4- cells are first transfected with a Tat expressing plasmid (pcDNA3.1+/Tat101) and infected with recombinant vaccinia viruses expressing either the HIV-1 (vPE16) or HIV-2 (vSC50) envelope glycoproteins (M.O.I.=1 PFU/cell). The cells are then added to TZM-bl cells (express the CD4, CCR5 and CCR4 receptors and luciferase) in the presence of the drugs under analysis at different concentrations. When cell-to-cell fusion (syncytia) occurs the Tat protein diffuses to the TZM-bl cells activating the expression of a reporter gene (luciferase).

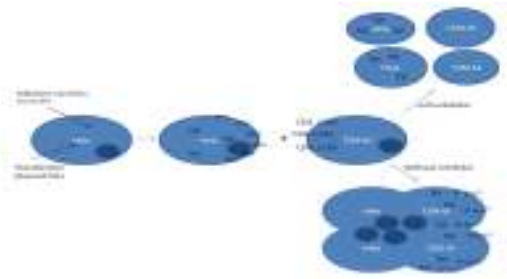

Figure 2 - Schematic representation of the novel method used to test cell-to-cell fusion inhibition

Results

We tested several entry inhibitors including the fusion inhibitors T1249, T20 and P3, the CCR5 antagonists Maraviroc and TAK-779, the CXCR4 antagonist AMD3100 and several neutralizing antibodies. All compounds inhibited HIV-1 and HIV-2 cell fusion albeit to different levels.

For HIV-1, the best cell fusion inhibitor was Maraviroc with an IC50 of 0.0076µM. T1249 and P3 had IC50s of 0.61µM and 1.34µM, respectively. TAK-779 was the weakest inhibitor, with an IC50 of 12.64µM. Maximum percentage of inhibition (MPI) by T20 was 75% at 10.02µM and by AMD3100 was 61% at 0.80µM. We are currently testing reference NAbs from HIV-1 infected patients for their cell fusion inhibition activity.

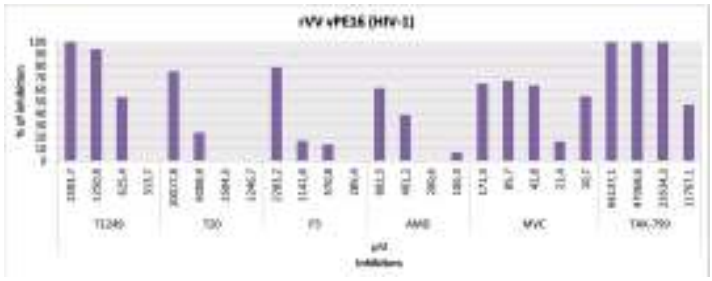

Figure 3 - % of inhibition of rVV vPE16 (HIV-1) with different concentrations of inhibitors.

For HIV-2, Maraviroc was also the best cell fusion inhibitor (IC50= 0.0603 µM) and T20 the worst (IC50 of 3.86µM). MPI by P3 was 95% at 1.42 µM, by AMD3100 was 45% at 0.80µM, by T1249 was 99.8% at 5µM and by TAK-779 was 55% at 23.20µM. The NAbs from HIV-2 infected patients we have tested so far did not prevent cell fusion.

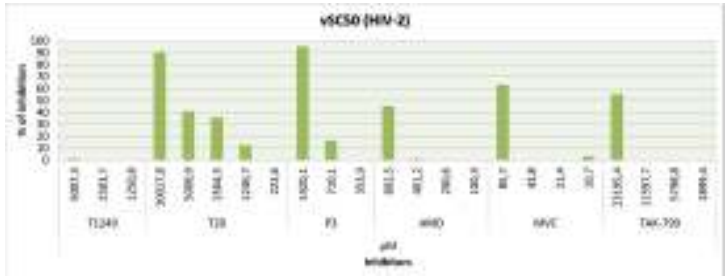

Figure 4 - Percentage of inhibition of vSC50 (HIV-2) with different concentrations of inhibitors

Cell fusion inhibition requires higher concentrations of inhibitors for HIV-1 and HIV-2, than cell free infection.

Table 1 - IC50 values for HIV-1 and HIV-2 cell free and cell fusion inhibition of infection

Inhibitor	Cell free IC50 (µM)		Cell fusion IC50 (µM)		Ratio cell fusion IC50/cell free IC50	
	HIV-1	HIV-2	HIV-1	HIV-2	HIV-1	HIV-2
T1249	0.0010	0.0043	0.6121	-	306.1	-
T20	0.0012	0.2815	-	3.8602	-	13.7
P3	0.0110	0.0638	-	-	132.4	-
AMD3100	0.0021	0.0026	-	-	-	-
MVC	0.0017	0.0021	0.0076	0.0603	4.4	28.7
TAK-779	0.0233	0.0199	12.6402	-	542.5	-

Conclusions

- HIV replicates more efficiently and rapidly through direct contact between cells, and this mode of transmission likely mediates a significant fraction of viral spread and immune evasion *in vivo*.
- This form of dissemination appears to be less susceptible to inhibition by antiretroviral drugs than cell-free virus transmission.
- Fusion and entry inhibitors in clinical use are much more effective at preventing cell-associated HIV-1 entry than HIV-2.
- Our new method will be useful to quickly identify new drugs and antibodies that can prevent cell-to-cell HIV-1 and HIV-2 infection.

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