

HIV-1 Entry Inhibitors Block Cell-to-Cell Infection in an *in vitro* Model of the Human Placental Barrier

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BACKGROUND

Remarkable Progress has been made to prevent HIV-1 mother-to-child transmission (PMTCT) in resource-limited settings. However, viral resistance to Nevirapine®, the first line drug used in most national PMTCT programs, remains a critical issue. Thus, new antiretroviral drugs and regimens must be identified and validated for PMTCT.

OBJECTIVES

To evaluate *in vitro* the efficacy of HIV-1 entry inhibitors to block cell-to-cell infection and the passage of the virus at the materno-fœtal interface.

MATERIAL AND METHODS

Material

Cells:

Human PBMCs from normal healthy donors and the human placenta trophoblast derived cell line BeWo (ATCC CCL98) expressing or not surface CD4 molecule were used.

Viruses:

The following HIV-1 isolates were used: HIV-1 Ba-L (R5), HIV-1 A204 (R5X4), HIV-1 LAI (X4) and HIV-1 CALM551 (X4).

Antiretroviral drugs:

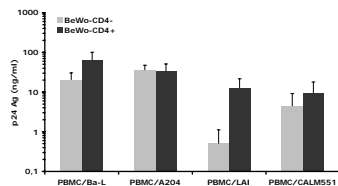
The CCR5 inhibitors TAK779 (NIH), SCH-350581 (kind gift of Schering Plough Research Institute), the CXCR4 antagonist AMD3100 (NIH) and the fusion inhibitors T20 and C34 (NIH) were used.

Methods

We used the Transwell® double chamber system where BeWo cells (CD4- or CD4+) were grown as a tight and polarized monolayer of cells. They were used as target cells for infection with PBMCs infected by different HIV-1 isolates (effector cells) in the presence or not of entry inhibitors. The effector/target ratio was set to 2. Viral passage across the monolayer was checked by HIV-1 p24 Ag produced by indicator PBMCs at the basolateral side of the Transwell®, 8 days post-contact.

RESULTS

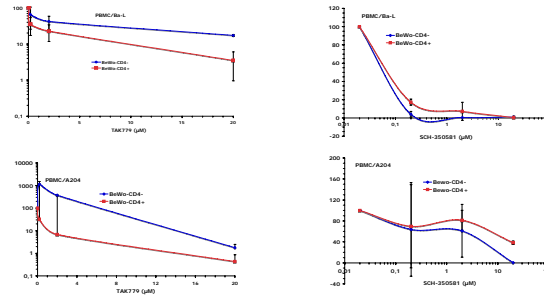
1. Significant viral passage after contact between BeWo cells (CD4- or CD4+) monolayer and PBMCs infected with HIV-1 isolates of various phenotypes.



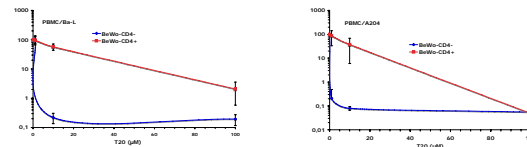
Legend to the figure:

The figure shows HIV-1 p24 Ag produced by indicator PBMCs after viral passage following target BeWo cells interaction with effector PBMCs infected with the different HIV-1 isolates indicated.

2. The CCR5 inhibitors TAK779 and SCH-350581 Efficiently block viral passage through the BeWo monolayer after contact with PBMC infected by R5 and R5X4 HIV-1.



3. The fusion inhibitor T20 Inhibits the passage of the virus through the BeWo monolayer in contact with PBMCs infected by R5 and R5X4 HIV-1.



4. The Fusion inhibitors T20 and C34 enhance the passage of the virus after contact between BeWo cells monolayer and HIV-1 X4 infected PBMC.

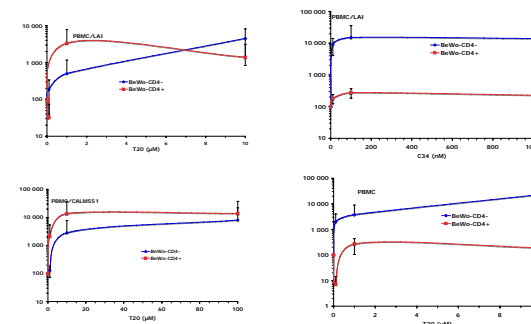
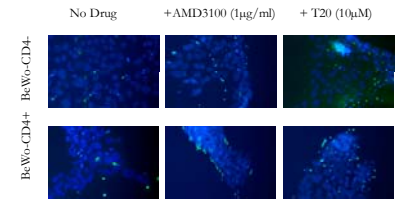


Table 1: 50% Inhibitory Concentrations of the Entry Inhibitors In HIV-1-PBMC/BeWo Cells Interactions

PBMC infected with:	HIV-1 Ba-L(R5)		HIV-1 A204 (R5X4)		HIV-1 LAI (X4)		HIV-1 CALM551 (X4)	
	CD4-	CD4+	CD4-	CD4+	CD4-	CD4+	CD4-	CD4+
T20 (µM)	5	30	3	8	Enhancement		Enhancement	
TAK779 (µM)	0.15	0.1	5	0.1				
SCH350581(µM)	0.09	0.11	5	15				
AMD3100 (µg/ml)	>1	>1	0.4	0.9	0.005	0.009	>0	>30

5. The Fusion Inhibitor T20 Enhances fusion between HIV-1/LAI (X4) infected PBMCs and non polarized BeWo Cells.



Legend to the figure:

HIV-1 LAI infected PBMCs (day 7) were loaded with the cytoplasmic dye Calcein-AM and used as effector cells against BeWo cells seeded on slides. Cell nuclei were counterstained with DAPI. The images (20X magnification) show the enhancement effect of T20, specially visible in BeWo CD4- cells with a massive green stain transfer from effector to target cells. A control with CXCR4 inhibitor is displayed and the efficacy (No entry) of the drug is clearer on BeWo CD4+ cells.

DISCUSSION

The present study shows that:

•CCR5 antagonists TAK779 and SCH-350581 inhibit the passage of the virus through the human placenta derived cell line BeWo in contact with PBMCs infected with R5 and dualtropic R5X4 HIV-1 isolates. The half maximal inhibitory concentrations for both molecules are in the micromolar range. The two drugs seem to act differently to block infection, as TAK779 is surprisingly more effective when target cells (BeWo) express surface CD4, while SCH-350581 is more effective when BeWo cells were not expressing CD4 on their surface. The two drugs fail to inhibit infection of BeWo cells with X4-HIV-1 infected PBMCs, as expected.

•The CXCR4 antagonist AMD3100 efficiently inhibits the passage of the virus through the BeWo cells monolayer in contact with HIV-1/X4 infected PBMCs with half maximal concentrations in the range of 5 to 800 ng/ml, depending on the viral isolate. PBMCs have been infected with. Whether a CXCR4 antagonist will be used for HIV positive patients' care and/or PMTCT remains a matter of debate as AMD3100 has been shown to be a powerful mobilizer of CD34+ progenitor cells.

•The fusion inhibitor T20 inhibits the the passage of the virus through the BeWo cells monolayer in contact with PBMCs infected with R5 and R5X4 HIV-1. The inhibition was dose-dependant with half maximal concentrations in the micromolar range. This is 250 and 1,000 folds higher than the IC50s we observed in the classical system with free virus/PBMC infection (not shown).

•Surprisingly, T20 (and C34) actually enhance the passage of HIV-1/X4 across the BeWo cells monolayer. Up to 1,000 fold increase from baseline was observed. This enhancement is dose-dependent, CD4 independent and co-receptor dependent. Enhancement by T20 occurs with non polarized target cells and is likely an enhancement of fusion capacity of the two cell membranes, as it does not occur with free viral particle.

•In conclusion, our data encourage further evaluation of CCR5 antagonists in their potency to prevent HIV-1 transmission across the placental barrier. More studies and much attention should be paid for the fusion inhibitor T20 in its use at the foeto-maternal interface.

Acknowledgements

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