Entry Inhibitors and Carbosilane Dendrimers are Potent Inhibitors of Cell-free and Cell-Associated HIV-2 Infection

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Background

Pre-exposure prophylaxis with topical microbicides formulated with antiviral agents can prevent HIV-1 acquisition. The ideal microbicide should prevent both cell-free and cell-associated HIV-1 and HIV-2 infections in the vaginal or rectum mucosa. So far, few candidate microbicides have been tested against HIV-2. Previously, we have shown that anionic carbosilane dendrimers 2G-S16 (sulphonate), G2-NF16 (naphthylsulfonated) and G3-S16 (sulphated-end groups) have potent antiviral activity against HIV-1. In this work, we evaluated the activity of these dendrimers and the fusion inhibitors T-1249 and T20 in preventing cell-free and cell-associated HIV-2 infection.

Aims

To evaluate the activity of dendrimers 2G-S16, G2-NF16 and G3-S16 and fusion inhibitors T1249 and T20 against cell-free and cell-associated HIV-2 infection.

Methods

- The activity of the dendrimers on HIV-2 was evaluated at the following levels: virus inactivation, virus-cell attachment, virus interaction with CD4, CCR5 and CXCR4, and viral entry.
- Cell-to-cell fusion inhibition was tested in Hela and TZMbl cells using a novel method which combines recombinant vaccinia virus (rVV) expressing the syncytium inducing HIV-2 ISY envelope glycoprotein and a Tat expressing plasmid.

Results

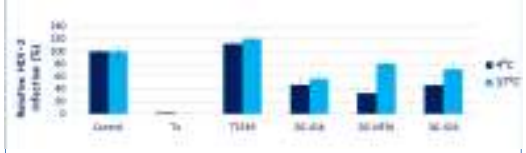
Cytotoxicity assay of the dendrimers in peripheral blood mononuclear cells (PBMCs) showed a maximum safe concentration of 1000µM, 50µM and 60µM for 2G-S16, G2-NF16 and G3-S16, respectively.

Results

Dendrimers inhibited 82 to >90% of HIV-2CBL-20 infectivity in PBMCs (82% for 2G-S16; >90% G2-NF16; 83% G3-S16) at a 10µM concentration with cell viability above 80%.

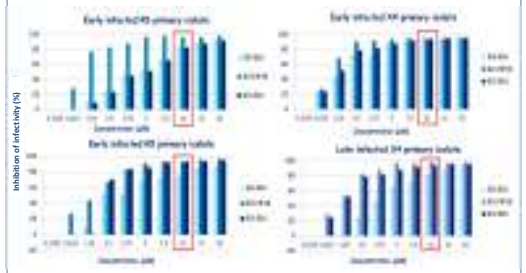
HIV-2 infection was determined using a capsid antigen assay [INNOTEST HIV Antigen mAb (Innogenetics)]

Dendrimers partially block virus attachment (55%, 62% and 52% with 2G-S16, G2-NF16, G3-S16 respectively) and entry to PBMCs (45%, 2G-S16; 20%, G2-NF16; 27%, G3-S16).

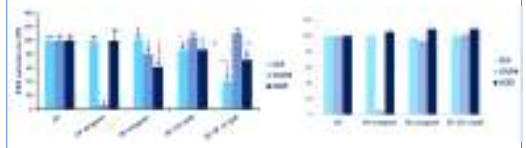


Virus attachment was evaluated at 4ºC; virus entry was evaluated at 37ºC.

In TZM-bl cells, at a 10µM concentration, dendrimers inhibit infectivity of primary R5 viruses by 70-95% and X4 isolates by 80-95%.



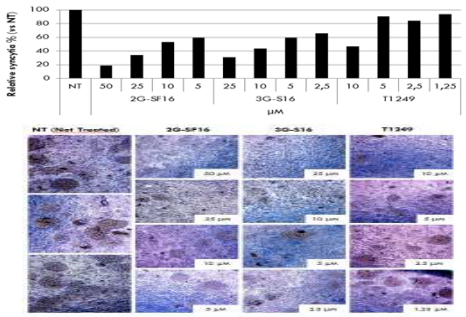
G3-S16 showed 15% binding to CD4 and CCR5. G2-NF16 showed 60% binding to CD4 and 29% binding to CCR5. 2G-S16 did not bind CD4 and CCR5. None of the dendrimers bind CXCR4.



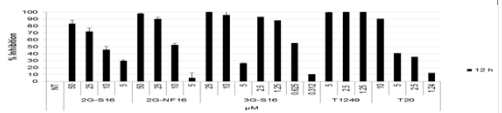
The competition experiments were performed by flow cytometry using antibodies targeting each of the receptors. FMI=fluorescence median intensity.

Results

All compounds reduced the number of syncytia.



All compounds tested inhibit cell-to-cell fusion in the luciferase assay. T1249 showed 100% inhibition for the concentrations tested.



Inhibition of cell-to-cell infection requires higher concentrations of all compounds than inhibition of cell-free infection.

Dendrimers	Cell-free IC50 (nM)	Cell-to-cell IC50 (nM)	Ratio (cell-to-cell/cell-free)
2G-SF16	2.97	13.21	8.4
2G-NF16	0.88	7.34	4.4
3G-S16	1.33	5.92	4.4

Conclusions

- 2G-S16, G2-NF16 and G3-S16 dendrimers inhibit cell-free HIV-2 infection by blocking the attachment and entry steps.
- Dendrimers inhibit HIV-2 syncytia formation suggesting that they also inhibit cell-to-cell infection
- Cell-to-cell infection is more difficult to inhibit by dendrimers than cell-free virus infection
- The data suggests that these dendrimers may be good microbicide candidates to prevent HIV acquisition.

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