Antiviral activity of 5-Hydroxytyrosol, a microbicidal candidate

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Introduction

5-Hydroxytyrosol (5-HT. Figure 1) is a natural compound that has previously shown biochemical activity against HIV integrase and gp41 (1,2). In this work we show that 5-HT is able to diminish viral replication without toxic effects in vitro

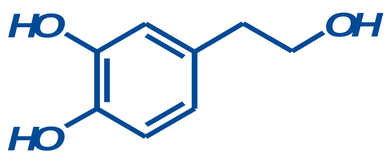


Figure 1. Chemical structure of 5-Hydroxytyrosol (5-HT)

Materials and methods

Recombinant viruses carrying luciferase-renilla reporters (Figure 2) with different properties (Wild type R5 and X4 tropic HIV, VSV pseudotyped HIV or resistant HIV clones) were used to infect a cell line (MT-2) or primary lymphocytes (PBMCs). Different concentrations of 5-HT were used in each assay to determine its potency and toxicity (3).

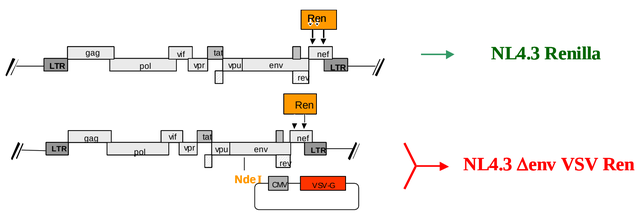
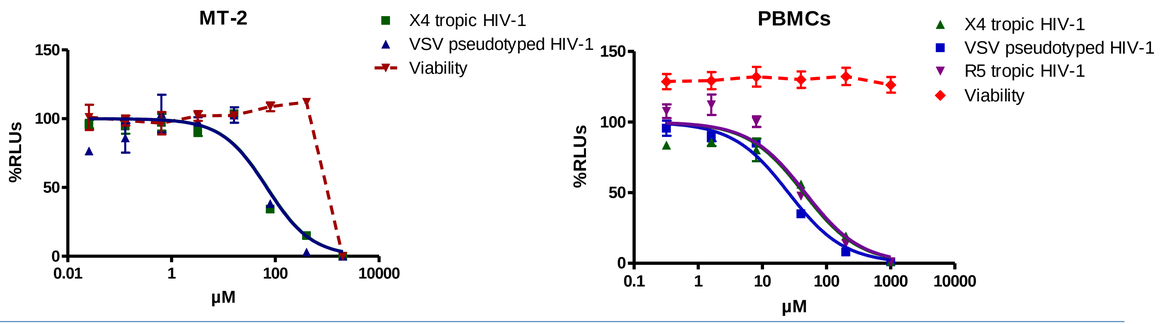


Figure 2. Scheme of the recombinant virus NL4.3-Renilla and NL4.3-Δenv-VSV-Ren. NL4.3 backbone (X4 tropic) was used as template and luciferase or renilla genes was inserted instead of *nef* gene. Recombinant HIV-1 with different properties (R5 tropic, VSV pseudotyped or Raltegravir resistant HIV-1) were obtained inserting the R5 *env* gene instead of NL4.3 *env* gene or co-trasnfecting pNL4.3-delta-env-Luc with VSV g protein. Raltegravir resistant HIV-1 were obtained by site directed mutagenesis.

Results

5-HT inhibited X4 and R5 tropic HIV infections in both MT-2 cells and primary lymphocytes (PBMCs) with IC_{50}s values between 30 and 80 µM.

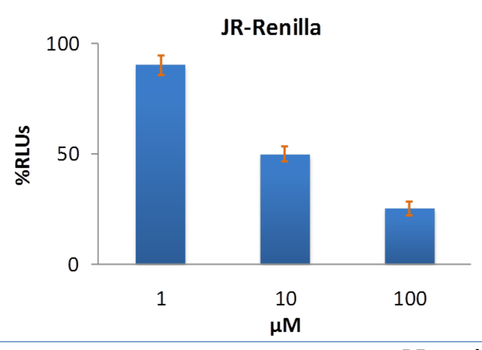


	X4 tropic HIV-1	VSV pseudotyped HIV-1	R5 tropic HIV-1	Cell Viability
MT-2				
IC_{50} µM	**80,07**	**92,96**	**ND**	**NR**
95% Confidence Intervals	42.97 to 149.20	39.10 to 221.00		
R^2	0,9298	0,8724		
PBMCs				
IC_{50} µM	**56.88**	**30.01**	**36.08**	**NR**
95% Confidence Intervals	32.00 to 101.10	20.29 to 44.39	24.04 to 54.16	
R^2	0.9524	0.9771	0.9753	

Figure 3. . Evaluation of the anti-HIV activity of hydroxytyrosol (5-HT) in MT-2 cells (left graph) and PBMCs (Right graph). MT-2 cells were infected with wild type X4 tropic recombinant virus NL4.3-Renilla or with VSV-pseudotyped HIV NL4.3-VSV-Luc. PBMCs were infected with wild type X4 tropic recombinant virus NL4.3-Renilla, with VSV-pseudotyped HIV NL4.3-VSV-Luc or with R5 tropic recombinant virus JR-Renilla. Cell viability was measured in the same conditions as infections, but without virus infection. Data obtained were analysed using GraphPad PRISM software (non linear regression) and IC_{50} values, Confidence interval 95% and R^2 are provided in the table (lower panel). IC_{50}: Inhibitory Concentration 50; ND: Not determined. NR: Not reached.

5-HT displays anti-HIV activity in both, MT-2 and PBMCs infections. Moreover, 5-HT is able to diminish viral replication in infections performed with X4, R5 and VSV pseudotyped HIV, suggesting a mechanism of action independent of the viral entry.
IC_{50} values are similar in all the infections. However, 5-HT seems to be more potent in PBMCs (IC_{50} values between 30 and 60 µM) than in MT-2 infections (IC_{50} values of 80 and 93 µM) and its activity is not due to cell death, since it lacks of cell toxicity at 1000 µM in PBMCs and just at this high concentration (1000 µM) reduces cell viability in MT-2.

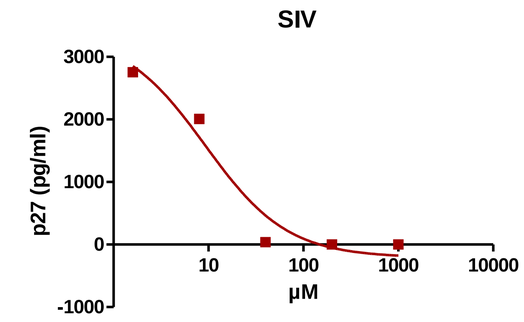
Iinhibition of DC-SIGN mediated trans-infection were also evaluated. DC-SIGN+ cells, such as dendritic cells (DCs), are antigen presenting cells (APCs) with a predominant role in the development of infection in vivo. To study the effect of HTS in this infection, we used a DC-SIGN+ cell line, RAJI-DC-SIGN as APCs and pre-activated PBMCs as target of the infection (Figure 4).



	R5 tropic HIV-1
RAJI DC-SIGN-PBMCs	
IC_{50} µM	4.88
95% Confidence Intervals	2.82 to 8.46
R^2	0.9867

Figure 4. DC-SIGN mediated trans-infection inhibition of 5-HT. RAJI DC-SIGN cells were treated with 5-HT to allow adsorption and 1 hour later viral supernatants (R5 tropic HIV-1) were added to cell culture. Afterwards, cells were harvested and cultures in 96-well microplates and treated again with the same concentrations of HTS (100, 10 and 1 µM) in the presence of preactivated PBMCs. 48 hours later, cell culture were lysed and luciferase activity measured in a luminometre.

When antigen presenting cells (APCs) DC-SIGN+ were present in cell culture, 5-HT anti-HIV potency was >10 fold higher, which would be essential for its microbicide activity, since mucosae infection is highly enhanced by APCs.

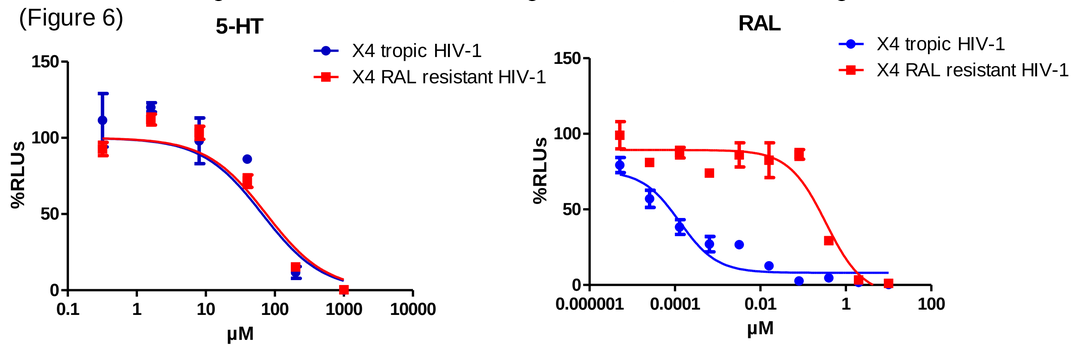


	SIV
Hydroxytyrosol (5-HT)	
IC_{50} µM	**9,247**
95% Confidence Intervals	0.4025 to 212.4
R^2	0,9556

Figure 5. CEM cells were infected with the strain SIV mac 251 at a dose of 500 TCIDs per 500.000 cells. Viral production was assessed by p27CA levels in culture supernatants at day 5 after infection using an ELISA assay (Zeptometrix corporation). A dose response to 5-HT (1000, 200, 40, 8 and 1.6 µM) in triplicate assays was analyzed. Estimated IC_{50} was 9µM which is In the range obtained against HIV-1. Data obtained were analysed using GraphPad PRISM software (non linear regression) and IC_{50} values, 95% Confidence intervals and R^2 are provided in the table (lower panel). IC_{50}: Inhibitory Concentration 50.

5-HT was also able to inhibit Simian immunodeficiency virus (SIV) replication as measured by ELISA detection of p27 SIV protein in cell culture infected supernatants (Figure 5).

5-HT was active against HIV-resistant viruses against NNRTI, NRTI and Integrase inhibitors (Figure 6)

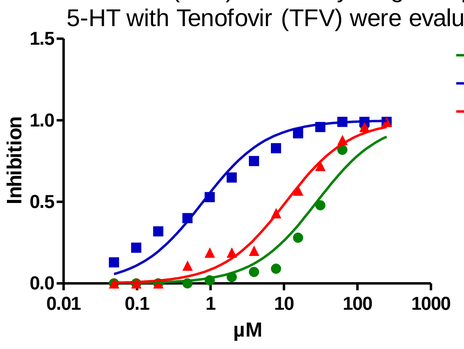


	X4 tropic HIV-1	X4 tropic RAL resistant HIV-1
Hydroxytyrosol (5-HT)		
IC_{50} µM	**65,97**	**75,29**
95% Confidence Intervals	27.04 to 161.0	48.29 to 117.4
R^2	0,8913	0,9311
Raltegravir (RAL)		
IC_{50} µM	**0,0001278**	**0,3397**
95% Confidence Intervals	6.021e-005 to 0.0002714	0.1857 to 0.6214
R^2	0,8761	0,9331

Figure 6. Evaluation of the anti-HIV activity of hydroxytyrosol (5-HT) in Raltegravir resistant HIV (NL4.3-148-Ren) infection of MT-2 cells . MT-2 cells were infected with wild type X4 tropic recombinant virus NL4.3-Renilla or with Raltegravir resistant HIV NL4.3-148-Ren. Data obtained were analysed using GraphPad PRISM software (non linear regression) and IC_{50} values, 95% Confidence intervals and R^2 are provided in the table (lower panel). IC_{50}: Inhibitory Concentration 50.

5-HT anti-HIV activity is similar in both infections, wild type HIV (NL4.3-Renilla) and raltegravir resistant HIV (NL4.3-148-Ren), while raltegravir inhibits raltegravir resistant HIV with IC_{50} values more than 50 fold higher than wild type HIV. Therefore, 5-HT will be active also in Raltegravir resistant HIV infections.

Lastly, an ideal microbicide would be composed by at least two different drugs. Since Tenofovir (TFV) is the only drug with positive results in clinical assays, combinations of 5-HT with Tenofovir (TFV) were evaluated.



Drug	CI Values at					
	ED50	ED75	ED90	Dm	m	r
5-HT	N/A	N/A	N/A	20,83	1,63	0,9842
TFV	N/A	N/A	N/A	0,60	0,77	0,9949
MIX 5-HT-TFV (70:2)	**0,68**	**0,73**	**0,88**	7,16	1,05	0,9614

Figure 7. . Evaluation of the anti-HIV activity of hydroxytyrosol (5-HT) combined with TFV (ratio 70/2) in MT-2 cells. MT-2 cells were infected with wild type X4 tropic recombinant virus NL4.3-Renilla . Data obtained were analysed using CalcuSyn software. CI: Combination Index: If CI < 1 antagonism, CI = 1 additive effect, CI < 1 Syunergic effect.

5-HT and TFV combination showed a synergic effect in MT-2 cells since combination index (CI) is below 1 (Figure 7). Thus, this combination could be used in vivo, ruling out a potential antagonism between them.

Conclusions

Although 5-HT potency is not in the highest range, it is active against HIV in a wide range of situations including resistant viruses and is devoid of toxicity at doses 100 times higher than IC_{50}. Moreover, 5-HT formulation as vaginal gel is easy and cheap and TFV/5-HT combinations shows a synergic effect in vitro. These characteristics make 5-HT a good microbicide candidate either alone or in combination with other antiretroviral drugs.

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Acknowledgments

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