Antiviral activity of 5-Hydroxytyrosol, a microbicidal candidate

L.M. Bedoya[1], M. Beltrán[1], D. Auñon[2], E. Gomez-Acebo[2], J. Alcami[1]

[1]Centro Nacional de Microbiología, Instituto de Salud Carlos III, AIDS Immunopathology, Majadahonda, Spain, [2]Seprox Biotech, Madrid, Spain

Contact AIM-HIV: AIM-HIV@isciii.es. Web: http://aim-hiv.isciii.es

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

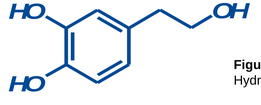
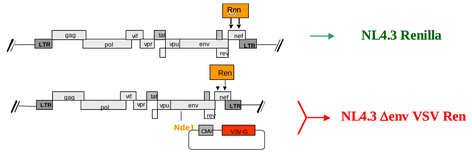


Figure 1. Chemical structure of 5-Hydroxytyrosol (5-HT)

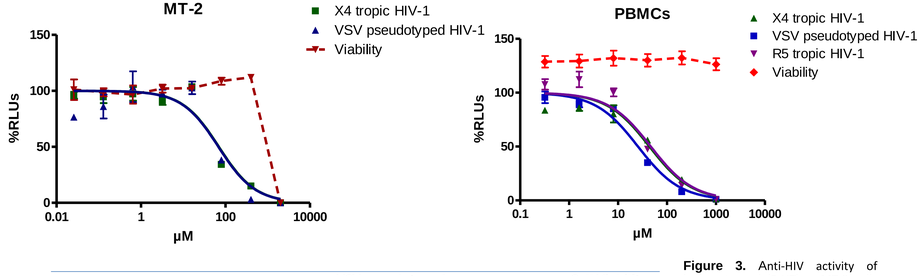
Materials and methods

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).



Resultados

5HT IS ACTIVE AGAINST WILD-TYPE HIV AND VSV PSEUDOTYPED VIRUS IN VITRO



	X4 tropic HIV-1	VSV pseudotyped HIV-1	R5 tropic HIV-1	Cell Viability CC50
MT-2				
IC_{50} µM	**80,07**	**92,96**	**ND**	**>500<2000**
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**	**>1000**
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. Anti-HIV activity of hydroxytyrosol (5-HT) in MT-2 cells (left graph) and PBMCs (Right graph) 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. 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 (Figure 3).

5-HT IS ACTIVE AGAINST HIV TRANSMISSION IN THE IMMUNE SYNAPSE

DC-SIGN+ cells, such as dendritic cells (DCs), are antigen presenting cells (APCs) with a predominant role in the development of infection in vivo. When DC-SIGN+ cells 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 (Figure 4).

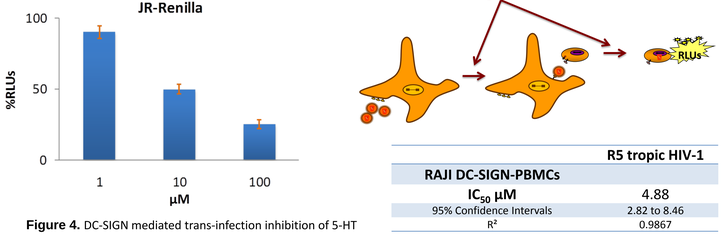
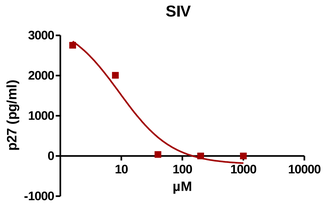


Figure 4. DC-SIGN mediated trans-infection inhibition of 5-HT

	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

5-HT IS ACTIVE AGAINST SIV IN VITRO



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

Figure 5. Evaluation of SIV inhibition by 5-HT.

5HT IS ACTIVE AGAINST RALTEGRAVIR-RESISTANT VIRUSES

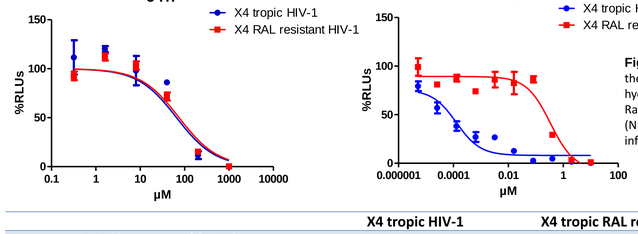
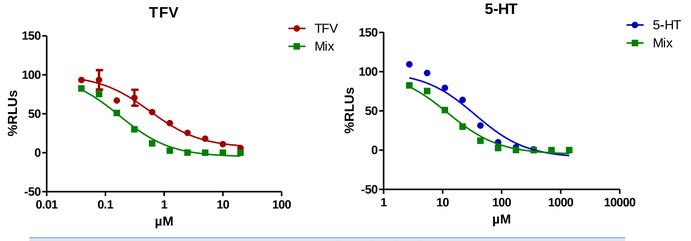


Figure 6. Evaluation of the anti-HIV activity of hydroxytyrosol (5-HT) in Raltegravir resistant HIV (NL4.3-148-Ren) infection of MT-2 cells .

	X4 tropic HIV-1	X4 tropic RAL resistant HIV-1
5-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

5HT DISPLAY A STRONG SYNERGSITIC EFFECT WITH TENOFOVIR IN VITRO



	Combination Index		
	ED50	ED75	ED90
PBMCs IL-2	0,307	0,252	0,390
PBMCs PHA/IL-2	0,237	0,223	0,380
MT-2	0,558	0,490	0,469

Figure 7. Evaluation of the anti-HIV activity of 5-Hydroxytyrosol (5-HT) combined with TFV (ratio 70/2) in MT-2 cells and PBMCs pre-activated with IL-2 (ratio 1/30) or with PHA/IL-2 (ratio 1/40). Data obtained were analysed using CalcuSyn software. Figure shows MT-2 combinations experiments.

CI: Combination Index.

CI >1.30 antagonism, 1.10–1.30 weak antagonism, 0.90–1.10 additive, 0.70–0.90 weak synergy, <0.70 strong synergy

5-HT and TFV combination showed a synergic effect in MT-2 cells, IL-2 and PHA/IL-2 pre-activated PBMCs since combination indexes (CI) are 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.

Acknowledgments

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