

ROLE OF CORECEPTOR INHIBITORS IN BLOCKING HIV-1 PRIMARY ISOLATES *IN VITRO*

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BACKGROUND

Different strategies in blocking HIV-1 entry are being evaluated either by targeting one of the cellular receptors, CD4 or coreceptor, or the envelope proteins. The majority of the available compounds interfere with the binding of gp120 with the coreceptor; these molecules are chemokine analogues, which block the in binding with the corresponding cellular receptor thus preventing the entry of HIV-1 in the target cells. Nowadays interesting results are obtained using anti-CCR5 monoclonal antibodies (MAbs).

OBJECTIVE

In this set of experiments we studied the effect on HIV-1 replication of several compounds directed against HIV-1 coreceptors in infections mediated by primary HIV-1 infection (PHI) isolates and by laboratory-adapted isolates. In particular we analyzed the efficacy of PA14, Tak-779, AOP-RANTES, Met-SDF-Fc and AMD-3100.

MATERIALS AND METHODS

Viral isolates

Some among our viruses were isolated from patients with primary HIV infection (PHI): 14aPre, DK were X4-tropic viruses; RM and MB were R5-tropic viruses.

Moreover, we used some V3 recombinant viruses constructed by inserting in the NL backbone V3 clonal sequences amplified from patients or viral strains: A4-3, B1-4, A2-I20F, A3-R23T, E4-E24G, AD8-2 (wild type), were R5 viruses; E1-6, B4-V20F, and SF2-2 (wild type) were dual tropic (R5-X4) viruses.

Drugs

We tested PA14 (Progenics Pharmaceuticals Inc, Tarrytown, NY, USA), a monoclonal antibody specifically targeted to CCR5, TAK-779 (Takeda Chemical Industries Ltd, Osaka, Japan), and Aminoxyptentane-(AOP)-RANTES (Gryphon Sciences, South San Francisco, CA, USA) directed against CCR5, AMD-3100 (AnorMED, Langley, BC, Canada) and Met-SDF-Fc (Genetic Institute, Cambridge, MA, USA) active against X4-tropic viruses.

PA14 was used at concentrations from 0,0625 to 0,5 $\mu\text{g/ml}$, TAK-779 from 0,011 to 0,3 μM , AOP-RANTES from 0,015625 to 0,125 $\mu\text{g/ml}$, AMD-3100 from 0,025 to 0,2 μM and Met-SDF-Fc from 0,0875 to 0,7 $\mu\text{g/ml}$.

Experimental design

All viruses were characterized for their tropism in CCR5- and CXCR4-transformed cell lines U87-CD4-CXCR4/CCR5, provided by Dr. Dan R. Littman (The Skirball Institute of Biomolecular Medicine, New York University School of Medicine, New York, NY) and sequenced in their env V3 region.

For each strain we conducted susceptibility tests according to an ACTG-modified protocol, using PA14, TAK-779, AOP-RANTES, Met-SDF-Fc, and AMD-3100 in order to determine the 50% and the 95% inhibitory concentration (IC_{50} and IC_{95}). Subsequently, we conducted experiments utilizing IC_{95} single or combined drugs against infections with RM (R5) or DK (X4) viruses or against mixed infections (1:1).

RESULTS 1

PA14 inhibited RM, an R5-tropic isolate, at IC_{50} of 0.028 $\mu\text{g/ml}$, whereas it did not show any activity in DK and 14aPre infections. TAK-779 was active versus RM and MB isolates: IC_{50} 0.0013 μM and 0.0014 μM , respectively; AMD-3100 was active versus DK and 14aPre isolates: IC_{50} 0.005 μM and 0.041 μM , respectively (Table 1).

Combination of AMD-3100 and TAK-779 inhibited dual infections with DK and RM (83.4%), whereas single drugs suppressed dual infections less efficiently (30.4-78.8%) (Table 2).

Table 1: Inhibitory concentrations (IC₅₀), of AMD-3100, TAK-779, and PA14 *versus* HIV-1 clinical isolates

	14aPre	DK	RM	MB
AMD-3100	0.041 μM	0.005 μM	Not inhibited	Not inhibited
TAK-779	Not inhibited	Not inhibited	0.0013 μM	0.0014 μM
PA14	Not inhibited	Not inhibited	0.028 $\mu\text{g/ml}$	n.d.

Note: 14aPre and DK are X4 strains, RM and MB are R5 strains.

n.d = not done

Table 2: Inhibitory efficacy of AMD-3100 and TAK-779 (IC₉₅) versus DK and RM.

Drugs	Viral isolates	p24(ng/ml)	% Inhibition
No drug	DK	7.25	
AMD-3100	DK	0.47	93.5
AMD-3100+TAK-779	DK	0.32	95.6
No drug	RM	7.25	
TAK-779	RM	1.75	75.9
AMD-3100+TAK-779	RM	1.45	80.0
No drug	DK+RM	10.85	
AMD-3100	DK+RM	7.55	30.4
TAK-779	DK+RM	2.30	78.8
AMD-3100+TAK-779	DK+RM	1.80	83.4

RESULTS 2

As regards the viruses derived from patients with a mutagenized R5 V3-loop we observed that PA14, AOP-RANTES, and TAK-779 inhibited these strains in a dose-dependent manner (Table 3). The only exception was represented by the inefficiency of PA14 against isolate A3-R23T.

We showed activity of AOP-RANTES (anti-R5) and Met-SDF-Fc (anti-X4) versus dual tropic chimerized isolates cultured in a R5/X4 cell mixture (Table 4 and Table 5).

Table 3: Inhibitory concentrations (IC₅₀) of AOP-RANTES, TAK-779, and PA14 versus HIV-1 R5-tropic laboratory-adapted strains

	AD8-2	A2-I20F	A3-R23T	A4-3	B1-4	E4-E24G
AOP-RANTES	0.030 µg/ml	0.003 µg/ml	0.009 µg/ml	0.012 µg/ml	0.010 µg/ml	0.027 µg/ml
TAK-779	0.050 µM	< 0.011 µM	0.008 µM	0.004 µM	0.008 µM	0.020 µM
PA14	0.277 µg/ml	0.207 µg/ml	> 0.5 µg/ml	0.480 µg/ml	0.178 µg/ml	0.221 µg/ml

Note: Viral input was 0.5 ng/0.5 ml of culture SN.

Table 4: Growth kinetics of HIV-1 X4/R5-tropic laboratory-adapted strains.

SF2-2		B4-V20F		E1-6	
PBMC	<i>X4/R5 mix</i>	PBMC	<i>X4/R5 mix</i>	PBMC	<i>X4/R5 mix</i>

day 7	1.4 ng/ml	1.1 ng/ml	0.6 ng/ml	0.2 ng/ml	0.8 ng/ml	1.6 ng/ml
day 11	3 ng/ml	1.7 ng/ml	0.5 ng/ml	1.8 ng/ml	0.7 ng/ml	1.7 ng/ml
day 14	1.6 ng/ml	1.1 ng/ml	0.2 ng/ml	1 ng/ml	0.2 ng/ml	1 ng/ml

Note: Antigen p24 values.

Table 5: Inhibitory concentrations (IC₅₀) of AOP-RANTES and Met-SDF-Fc *versus* HIV-1 X4-R5-tropic mutagenized strains in X4/R5 cell mixtures.

	SF2-2	B4-V20F	E1-6
AOP-RANTES	0.0156 μM	< 0.0156 μM	0.0156 μM
Met-SDF-Fc	0.014 μg/ml	0.059 μg/ml	0.071 μg/ml

Note: The isolates used were obtained from day 11 (X4/R5) cultures and the viral input was 0.5 ng/0.5 ml of culture SN.

CONCLUSIONS

Our experiments expanded previous findings and indicated a selective activity of these attachment/entry inhibitors against HIV-1 clinical isolates with a defined coreceptor use. Interestingly, we found that the compounds we tested were effective against a wide range of R5- or X4/R5-tropic mutagenized isolates.

The combined activity of R5- and X4- inhibitors may be useful in inhibiting mixed infections and warrants further analysis prior in vivo administration.

Selected readings

- Berger EA, Murphy PM, Farber JM. *Chemokine receptors as HIV-1 coreceptors: roles in viral entry, tropism, and disease*. *Ann Rev Immunol*, 1999; 17: 657-700.
- Lusso P. *Chemokines and viruses: the dearest enemies*. *Virology*, 2000; 273: 228-240.
- Rusconi S, La Seta Catamancio S, Citterio P, Bulgheroni E, et al. *Combination of CCR5 and CXCR4 inhibitors in therapy of human immunodeficiency virus type 1 infection: in vitro studies of mixed virus infections*. *J Virol*, 2000; 74: 9328-9332.
- Olson WC, Gwenaël E, Rabut E, et al. *Differential inhibition of human immunodeficiency virus type 1 fusion, gp120 binding, and CC-chemokine activity by monoclonal antibodies to CCR5*. *J Virol*, 1999; 73: 4145-4155.
- Dragic T, Litwin V, Allaway GP, et al. *HIV-1 entry into CD4+ cells is mediated by the chemokine receptor CCR5*. *Nature*, 1996; 381: 667-673.
- Deng H, Liu R, Ellmeier W, et al. *Identification of a major co-receptor for primary isolates of HIV-1*. *Nature*, 1996; 381: 661-666.
- Cocchi F, De Vico A, Garzino-Demo A. *Identification of RANTES, MIP-1 α and MIP-1 β as the major HIV-1 suppressive factors produced by CD8+ T cells*. *Science*, 1995; 270: 1811-1815.