Suppressive Effect of Polyoxometalates on the Cytopathogenicity of Human Immunodeficiency Virus Type 1 (HIV-1) *in Vitro* and Their Inhibitory Activity against HIV-1 Reverse Transcriptase

Yoshio INOUYE,*[,a] Yoshiki TOKUTAKE,[a] Junko KUNIHARA,[a] Tetsuya YOSHIDA,[b] Toshihiro YAMASE,[c] Atsuo NAKATA[d] and Shoshiro NAKAMURA[a]

Institute of Pharmaceutical Sciences[a] and Department of Microbiology,[b] Hiroshima University School of Medicine, Hiroshima 734, Japan, Research Laboratory of Resources Utilization, Tokyo Institute of Technology,[c] Yokohama, Kanagawa 227, Japan and Research Institute for Microbial Diseases, Osaka University,[d] Suita, Osaka 565, Japan. *Received September 9, 1991*

One isopolyoxometalate and 42 heteropolyoxometalates consisting of 3 compounds with the trivacant Keggin structure, 2 with the lacunary Keggin structure, 30 with the Keggin structure, one with the Wells-Dawson structure and 6 with miscellaneous structures were tested for their suppressive effect on the cytopathogenicity of human immunodeficiency virus type 1 (HIV-1) *in vitro* and inhibitory activity against HIV-1 reverse transcriptase.

In contrast to the leading interpretations which attribute the suppressive effect of polyoxometalates on the cytopathogenicity of HIV-1 to the inhibition of HIV-1 reverse transcriptase by these compounds, there was no distinct correlation observed between these two functions of polyoxometalates.

Keywords polyoxometalate; human immunodeficiency virus type 1 (HIV-1); reverse transcriptase; antiviral activity; enzyme inhibitor; acquired immunodeficiency syndrome (AIDS)

Polyoxometalate HPA 23, $(NH_4)_{17}Na[NaSb_9W_{21}O_{86}]\cdot 14H_2O$,[1] was found to be active against a broad spectrum of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) viruses *in vivo* and *in vitro*[2]: transformation of mouse embryonic fibroblasts by murine sarcoma virus, disease caused by Friend leukemia or Moloney murine sarcoma virus, encephalomyocarditis and vesicular stomatis virus infection in mice, rabies in mice and foxes and modification of Epstein-Barr virus early antigen expression in Raji cells treated with iododeoxyuridine. HPA 23 was also reported to be an inhibitor of various DNA and RNA polymerases of cellular, bacterial or viral origin.[3] Virus-coded reverse transcriptase plays a central role in the replication of retroviruses such as human immunodeficiency virus type 1 (HIV-1), a causative agent for acquired immunodeficiency syndrome (AIDS).[4] Besides reverse transcriptase of primate retroviruses, HIV-1 enzyme was inhibited by HPA 23 noncompetitively with respect to template-primer.[5] Human DNA polymerase α was also susceptible to the inhibitory activity of HPA 23 to a lesser extent than HIV-1 reverse transcriptase,[6] accounting for the preferred inhibition of the growth of HIV-1.

On the basis of these observations, three patients with AIDS and one with prodrome were treated with HPA 23.[7] Although HPA 23 treatment resulted in a pronounced decrease in reverse transcriptase activity in lymphocyte cultures of AIDS patients,[8] no improvement in the clinical or immunological status of the patients was observed.[9] In addition to these clinical failures, HPA 23 was found not to be able to protect $CD4^+$ cells, *i.e.*, ATH 8 and MT-4 cells, against the cytopathic effect of HIV-1 *in vitro*.[10, 11a]

The authors have been screening polyoxometalates for inhibitors of the cytopathogenicity of HIV-1 against MT-4 cells, and have demonstrated marked activity of polyoxometalate PM-19, $K_7[PTi_2W_{10}O_{40}]\cdot 6H_2O$.[11] The virus adsorption to membrane receptors or the penetration into cells via fusion mechanism appeared to be a more probable target of PM-19 than reverse transcription,[11b] even though PM-19 was as potent an inhibitor of HIV-1 reverse transcriptase as HPA 23. To verify this hypothesis, the correlation between the *in vitro* anti-HIV-1 activity of a series of polyoxometalates and their inhibitory activity against HIV-1 reverse transcriptase was examined.

Materials and Methods

HIV-1 reverse transcriptase was assayed according to the method of Hoffman *et al.* with some modifications[12] using recombinant HIV-1 reverse transcriptase.[13]

Assay for the anti-HIV-1 activity was based on the inhibition of virus-induced cytopathogenicity in MT-4 cells[14] (details will be published elsewhere). Briefly, MT-4 cells were infected with III_b variant of HIV-1, at a multiplicity of infection (moi) of 0.01 for 1 h at 37 °C. Cells were brought into wells of 96-well microtiter plates and incubated with individual test compounds for 6 d at 37 °C in a total volume of 200 μl. The cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) metabolic assay.[15]

HPA 23 was kindly donated by Prof. A. Tézé, Universite Pierre *et* Marie Curie, and $Na_3[PMo_{12}O_{40}]\cdot nH_2O$ (PM-102) was commercially obtained. Other polyoxometalates were prepared and purified according to the published methods.[16] The polyoxometalates are subcategorized into iso- and hetero-polyoxometalates depending on the existence of "heteroatom(s)" in the anion structures.

Results

The test compounds used in this study consist of one isopolyoxotungstate and 42 heteropolyoxometalates (Table I).

In the reverse transcriptase assay, the samples were dissolved in H_2O at 500 and 100 μg/ml without being sterilized and mixed with 9 times the volume of reaction mixture. Depending on the % of inhibition (%I) values at 10 (low concentration) and 50 μg/ml (high conc.), the following interpreting guidelines were set for the enzyme inhibitory activity: $\geq$80% at low conc., significant; $\geq$80% at high conc. but not at low conc., moderate; 50—79% at high conc., weak; and $<$50% at high conc., poor.

For the assay of anti-HIV-1 activity, samples were dissolved in RPMI-1640 medium supplemented with 10% fetal calf serum at 1600 μg/ml and passed through 0.2 μm membrane filter for the sterilization. The 50% cytotocidal concentration (CC_{50}) and the 50% effective concentration (EC_{50}) were defined as the concentrations of compound that reduced the cell number of the mock-infected MT-4 cells by 50% and gave HIV-1-infected cells 50% protection from the cytopathogenicity of HIV-1, respective-

TABLE I. Correlation between Anti-HIV-1 Activity and Inhibitory Activity against HIV-1 Reverse Transcriptase

Compound	Molecular formula	Anti-HIV-1 activity			Inhibition of HIV-1 RT (%)	
		MT-4 CC_{50}[a] (μg/ml)	MT-4/HIV-1 EC_{50}[b] (μg/ml)	TI_{50}[c]	Concentration	
					10 μg/ml	50 μg/ml
Isopolyoxotungstate						
PM-70	$K_6[W_7O_{24}]\cdot 8H_2O$	(>800)[d]	—[e]	—	0	0
Heteropolyoxometalates						
Trivacant Keggin structure, 1:9,[f] tetrahedral[g]						
PM-64	α-$Na_3H_6[PMo_9O_{34}]\cdot 13H_2O$	440	—	—	0	0
PM-30	A-β-$Na_9[SiW_9O_{34}H]\cdot 23H_2O$	120	28	4.3	0	12
PM-73	α-$Na_{10}[SiW_9O_{34}]\cdot 18H_2O$	120	9.9	12	23	76
Lacunary Keggin structure, 1:11, tetrahedral						
PM-65	α-$K_7[PW_{11}O_{39}]\cdot nH_2O$	180	—	—	0	0
PM-62	$K_7[PW_9Mo_2O_{39}]\cdot 19H_2O$	170	89	1.9	0	0
Keggin structure, 1:12, tetrahedral						
PM-63	$(NH_4)_6H[PZnMo_{11}O_{40}]\cdot 25H_2O$	(230)	—	—	0	0
PM-102	$Na_3[PMo_{12}O_{40}]\cdot nH_2O$	760	—	—	19	70
PM-67	$K_3[PMo_9W_3O_{40}]\cdot 5H_2O$	250	—	—	0	0
PM-66	$K_3[PMo_3W_9O_{40}]\cdot 25H_2O$	(330)	(186)	1.8	0	0
PM-78	$K_9[BTi_2W_{10}O_{40}]\cdot nH_2O$	(260)	(71)	3.7	95	100
PM-74	$K_7[PGe_2W_{10}O_{40}]\cdot nH_2O$	38	7.9	4.8	27	29
PM-81	$K_7[PSe_2W_{10}O_{40}]\cdot nH_2O$	280	—	—	5	93
PM-76	$K_7[PSi_2W_{10}O_{40}]\cdot nH_2O$	130	5.1	26	0	31
PM-75	$K_7[PTe_2W_{10}O_{40}]\cdot nH_2O$	<3.1	—	Toxic	4	5
PM-19	$K_7[PTi_2W_{10}O_{40}]\cdot 6H_2O$	270	4.0	68	15	62
PM-77	$(Me_4N)_7[PTi_2W_{10}O_{40}]\cdot nH_2O$	260	4.4	59	17	46
PM-82	$K_7[PV_2W_{10}O_{40}]\cdot nH_2O$	(220)	(6.4)	34	0	61
PM-79	$K_7[SiTi_2W_{10}O_{40}]\cdot nH_2O$	(300)	(13.5)	22	100	100
PM-46	$K_6[BVW_{11}O_{40}]\cdot nH_2O$	140	7.0	20	3	26
PM-47	$K_7[BVW_{11}O_{40}]\cdot nH_2O$	310	4.4	71	5	41
PM-61	$K_5[PTiW_{11}O_{40}]\cdot nH_2O$	(>1600)	(30)	>53	99	100
PM-44	$K_5[PVW_{11}O_{40}]\cdot 6H_2O$	270	29	9.3	19	98
PM-85	$K_5[SiAl(H_2O)W_{11}O_{39}]\cdot nH_2O$	140	4.0	35	37	51
PM-84	$K_5[SiCo(H_2O)W_{11}O_{39}]\cdot nH_2O$	77	3.4	23	15	26
PM-88	$K_5[SiCr(H_2O)W_{11}O_{39}]\cdot nH_2O$	90	8.3	11	9	9
PM-89	$K_5[SiCu(H_2O)W_{11}O_{39}]\cdot nH_2O$	130	8.2	16	0	0
PM-83	$K_5[SiFe(H_2O)W_{11}O_{39}]\cdot 14H_2O$	140	5.0	28	0	0
PM-87	$K_5[SiMg(H_2O)W_{11}O_{39}]\cdot nH_2O$	110	8.2	13	0	15
PM-90	$K_5[SiMn(H_2O)W_{11}O_{39}]\cdot nH_2O$	140	7.1	20	0	0
PM-40	$K_6[SiNi(H_2O)W_{11}O_{39}]\cdot 15H_2O$	43	6.8	6.3	22	59
PM-91	$K_5[SiSr(H_2O)W_{11}O_{39}]\cdot nH_2O$	130	7.6	17	0	2
PM-43	$K_5[SiVW_{11}O_{40}]\cdot nH_2O$	220	6.1	36	7	37
PM-86	$K_5[SiZn(H_2O)W_{11}O_{39}]\cdot nH_2O$	85	6.5	13	0	31
PM-1	$K_5[BW_{12}O_{40}]\cdot 15H_2O$	190	17.5	11	0	19
PM-80	$Na_3[PW_{12}O_{40}]\cdot nH_2O$	180	—	—	0	32
Wells-Dawson, 2:18, tetrahedral						
PM-29	$K_8[P_2Co(H_2O)W_{17}O_{61}]\cdot 18H_2O$	(39)	(11.5)	3.4	100	100
Miscellaneous						
PM-2	$K_{18}[KSb_9W_{21}O_{86}]\cdot nH_2O$	28	—	—	0	24
HPA 23	$(NH_4)_{17}Na[NaSb_9W_{21}O_{86}]\cdot 14H_2O$	5.7	—	—	4	77
PM-72	$(NH_4)_{18}[(NH_4)Sb_9W_{21}O_{86}]\cdot nH_2O$	12	—	—	0	4
PM-3	$K_{27}[KAs_4W_{40}O_{140}]\cdot nH_2O$	73	13.5	5.4	53	95
PM-69	$K_{15}H_3[Eu_3(H_2O)_3W_{24}O_{87}]\cdot 25.5H_2O$	150	—	—	0	4
PM-104	$(NH_4)_{12}H_2[Eu_4(MoO_4)(H_2O)_{16}(Mo_7O_{24})_4]\cdot 13H_2O$	300	4.4	68	14	29

a) The 50% cytocidal concentration. b) The concentration at which the compound protects MT-4 cells from the cytopathogenicity of HIV-1 by 50%. c) The ratio of CC_{50}/EC_{50}. d) The apparent value on the assumption that a sample is completely solubilized in the culture medium at 1600 μg/ml. e) The maximum inhibition is less than 50%. f) Stoichiometry of the heteroatom (central atom): peripheral atom. g) Shape of coordination around a central atom.

ly.[11] Some compounds were scarcely or slightly soluble in the culture medium at 1600 μg/ml. The EC_{50} and CC_{50} values of these compounds are given in parentheses because they are merely apparent values. However, the comparison of TI_{50} values (CC_{50}/EC_{50}) can be done without regard to the solubility of individual compounds.

All the potent inhibitors of HIV-1 reverse transcriptase, *i.e.*, PM-29, -61, 78 and -79, are difficult to completely solubilize in the culture medium. However, the reverse is not true; the compounds with poor solubility are not always potent inhibitors of the enzyme. As for the compounds referred to as moderate or weak inhibitors of HIV-1 reverse transcriptase, anti-HIV-1 activity varied enormously; the TI_{50} values ranged from "-(or not available)" to *ca.* 70. The anti-HIV-1 activity of PM-104 compares favorably with or superior to those of heteropolyoxotungstates. It is evident

that more than 60% of Keggin heteropolyoxotungstates as well as PM-104 are poor inhibitors of HIV-1 reverse transcriptase.

HPA 23 and its analogues (PM-2, -72) could not protect MT-4 cells from the cytopathogenicity of HIV-1. Although HPA 23 inhibited reverse transcriptase to some extent, it was highly toxic with CC_{50}, being as low as 5.7 μg/ml.

Discussion

Interference with reverse transcription has been documented as the mechanism by which HPA 23 inhibits the growth of retroviruses including HIV-1. Hervé *et al.*[3c] reported correlation between the structure of polyoxotungstates and their inhibitory activity on Moloney murine leukemia virus reverse transcriptase using tungstosilicate (TS) $[SiW_{11}O_{39}]^{8-}$; tungstoantimoniates (TA) $[XSb_9W_{21}O_{86}]^{19-}$, where X is Na^+, K^+, Ca^{2+} or Sr^{2+}; and tungstoarsenates (TAs) $[XAsW_{40}O_{140}]^{28-}$, where X is Na^+, K^+ or Ba^{2+}. The magnitudes of inhibition of reverse transcriptase by these compounds were in the order of TS<TA<TAs. Further, TAs was less toxic than TA; however, TAs did not show antiviral activity *in vivo*.

HPA 23 is a member of the TA family with potassium ion in the central cage and PM-3 is TAs with potassium ion in the central cavity. Compared with PM-3, HPA 23 was more cytotoxic and a less potent inhibitor of reverse transcriptase; these results were in good agreement with the previous findings of Hervé *et al.*[3c]

The EC_{50} values for most of the polyoxotungstates with potent anti-HIV-1 activity, *e.g.*, PM-19 and -104, ranged between 4 and 9 μg/ml and the CC_{50} values were higher than 100 μg/ml, whereas CC_{50} for HPA 23 was as low as 5.7 μg/ml (Table I). The protection of MT-4 cells from the cytopathogenicity of HIV-1 by HPA 23 might, therefore, be masked by its cytotoxicity. PM-3 was less toxic than HPA 23 and showed some protective effect on MT-4 cells. However, TAs reportedly did not exhibit antiviral activity *in vivo*.[3c] Taking these results into consideration, the antiviral activity of TA in animal models[3c] was considered to be the result of cytotoxicity.

Even though striking inhibition of reverse transcriptase was shown by some polyoxometalates, this could not account for their anti-HIV-1 activity, because it is clearly shown in Table I that there is no correlation between anti-HIV-1 activity and inhibitory activity against reverse transcriptase.

These results are not incompatible with our previous findings[11b] that certain heteropolyoxometalates interfered with HIV-1 infection at very early stages such as adsorption to membrane receptors and/or penetration into target cells.

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