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In vitro antiviral activity of polyoxotungstate (PM-19) and other polyoxometalates against herpes simplex virus

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Summary

Polyoxotungstates with Keggin-type structure were found to demonstrate marked antiherpetic activity. $K_7[Ti_2W_{10}PO_{40}]\cdot 6H_2O$ (PM-19) caused a decrease in plaque formation by several strains of herpes simplex virus (HSV) type 1, including acyclovir-resistant (thymidine kinase-negative) strains, at concentrations which were not toxic to the host cells. The 50% plaque-inhibiting concentration (EC_{50}) for the different strains was between 20 and 50 μg/ml. Single-cycle HSV growth was also inhibited by PM-19. PM-19 inhibited viral DNA synthesis in HSV-infected cells at a concentration of 5 μg/ml but did not exhibit a virucidal effect, and pretreatment of the host cells with PM-19 did not provide resistance to herpes infection. Yet, virus adsorption to the cells was markedly affected at PM-19 concentrations higher than 25 μg/ml. PM-19 was also effective against human cytomegalovirus, but not against adenoviruses and varicella-zoster virus, although it did delay the development of the cytopathic effect of these viruses.

Polyoxometalate; PM-19; Keggin-type structure; Antiherpetic agent

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Introduction

Heteropolyanions are a class of polynuclear coordination complexes, formed particularly by the transition metals of the vanadium and chromium series. Some heteropolyanions have been reported to exert a strong inhibitory effect on RNA and DNA viruses in plants and animals (Jasmin et al., 1973; Agrawal et al., 1981; Sharma et al., 1984) and to inhibit DNA and RNA polymerases from various microorganisms and mammal species (Haapala et al., 1973; Hervé et al., 1975,1983). They are also known to inhibit reverse transcriptase of the human immunodeficiency virus (Dormont et al., 1988). Similarly, some of these heteropolyanions have also been shown to inhibit the growth of transplanted tumors in experimental animals (Yamase et al., 1988).

As heteropolyanions may assume a wide variety of structures depending on the ratio of the central and the peripheral ions, we have tested several heteropolyanionic types for their activity against some DNA viruses, and found that Keggin-type polyoxometalates exhibit marked activity against several strains of herpes simplex virus and some DNA and RNA viruses (Fukuma et al., 1989; Dan et al., 1990; Yamase et al., 1991). In this report we describe the antiherpetic effects of several polyoxometalates, with special emphasis on one of these compounds, $K_7[Ti_2W_{10}PO_{40}]\cdot 6H_2O$ (PM-19).

Materials and Methods

Viruses

The strains of herpes simplex virus (HSV) type 1 used in this study were as follows: KOS and Tomioka, provided by Dr. T. Kurata (Institute of Medical Science, University of Tokyo); Hayashida, provided by Dr. R. Mori (Kyusyu University); R6′ and R8′, which are acyclovir (ACV)-resistant strains isolated from the Hayashida strain. These resistant strains are thymidine kinase-negative, and about 125-fold less susceptible to ACV than the parental strain. Other viruses studied were HSV type 2 (196 strain), the human cytomegalovirus (HCMV, AD169 strain), adenovirus (types 1 and 5), and vaccinia virus (IHD strain).

Cells

The Vero cells and human embryonic lung cells (HEL; Flow 2000 cells) used were purchased from Flow Laboratories Inc, U.S.A., and cultured in Eagle's minimum essential medium (MEM; Nissui Pharmaceutical Co. Inc.) supplemented with 10% fetal bovine serum (FBS; Flow Laboratories Inc.).

Chemicals

Polyoxometalates were synthesized according to the procedures that have been described elsewhere (Yamase et al., 1977,1986; Ozeki et al., 1991; Domaille et al., 1983; Jasmin et al., 1973). Acyclovir was provided by The Wellcome Foundation Ltd., U.K. Phosphonoacetic acid and 9-β-D-arabinofuranosyladenine (Ara-A) were purchased from Sigma Chemical Co.

Cytotoxicity test

Monolayers of Vero cells were treated with various concentrations of each compound for 5 days. The cells were examined microscopically. The highest concentration which caused no cytotoxic effect was estimated to be the maximum nontoxic dose (MNTD). The growth inhibitory effect was assessed in Vero cells in 24-well multidish trays. Exponentially growing Vero cells were treated with the compounds at various concentrations for 72 h, then the cells were dispersed with trypsin and counted by a hemocytometer. The cytotoxic effect of the compounds in HEL cells was assessed by a colorimetric (MTT) assay (Chemicon International Inc., U.S.A.). Monolayers were treated with compounds at various concentrations for 72 h and were then processed according to the instructions given with the MTT assay kit. The absorbance was measured on an ELISA plate reader (model MPR A4; Tosoh Co., Japan) with a test wavelength of 570 nm and a reference wavelength of 630 nm.

Assay of antiviral activity

Antiviral activity was determined by the reduction of a 50% tissue-culture-infective dose ($TCID_{50}$). HSV type 1 (Hayashida strain) and type 2 (196 strain) were used. Ten-fold dilutions of the stock virus preparation were incubated with Vero cells grown in 96-well microplates (Nunc, Denmark). MNTDs (the highest concentrations that caused no morphological change in host cells cultured for five days) or half of the MNTD of each compound were then added and the cytopathic effect (CPE) was recorded for five days using an optical microscope. With the polyoxometalates, whose MNTDs were greater than 200 μg/ml, 200- and 100-μg/ml amounts were employed as the test concentrations. The virus stock dilution that infected half of the wells was estimated as a $TCID_{50}$. Antiviral activity was judged to be positive when the difference in $TCID_{50}$s between the compound-treated and nontreated control cells was more than 1.0 $\log_{10}$. Usually the $TCID_{50}$ value of the control group ranged between 10^{-7} and $10^{-7.5}$. The reduction in a $TCID_{50}$ value >3.0 meant that no CPE was observed even when 1000 $\times$ the $TCID_{50}$ of the virus was used.

The antiviral activity was also evaluated by a plaque assay. Vero cells grown in plastic dishes (35 mm in diameter, Nunc) were infected with about 50 PFU (plaque forming units) of virus. After a 1-h virus adsorption period, the cells

were covered with MEM containing various concentrations of polyoxometalates and 0.6% agarose (Seakem Inc., U.S.A.). The cells were cultured for 72 h, after which they were stained with neutral red and the number of plaques were counted.

The effect on the single cycle growth of HSV was examined by estimating the virus titer at 20 h after infection with a multiplicity of infection (MOI) of 0.1 PFU per cell. Various concentrations of each compound were added after a 1-h virus adsorption period. Twenty hours later, the culture fluid was collected as the extracellular fraction. The remaining cells were washed with phosphate-buffered saline (PBS). Hank's balanced salt solution (HBSS), supplemented with 1% bovine plasma albumin (BPA), was added. The cells were then disrupted by sonication and centrifuged, and the resulting supernatant was collected as an intracellular fraction.

Viral DNA synthesis

HEL cells were infected with the Hayashida strain of HSV-1 at different MOIs. After 1 h of virus adsorption, the cells washed with PBS, and maintenance medium containing various concentrations of polyoxometalates was added to the cells after which they were cultured for more than 4 h. The cultures then were pulse-labeled with 1 μCi/ml of [*methyl*-^{3}H]thymidine (specific activity 74.0 GBq/mmol; NEN Research Products) for 30 min. Immediately thereafter, the cells were washed with cold PBS, and the DNA was extracted by a method previously described by Davis et al. (1986). Briefly, the infected cells were treated overnight with proteinase K (1 mg/ml) containing 4% sodium dodecylsulfate, after which the DNA was extracted with phenol-chloroform. The extracted DNA was dissolved in a cesium chloride solution and centrifuged at 60 000 rpm at 20°C for 6 h in a Beckman TL-100 ultracentrifuge (rotor TLA-100.3). Fractions were collected (10 drops) from the bottom of the gradients, and the DNA was precipitated onto glass microfilters (GF/C, Whatman International Ltd., U.K.). The filters were dried and the radioactivity then assessed by a liquid scintillation counter LS9800 (Beckman Instruments Inc.).

The polyoxometalates were examined for their inhibitory effect on incorporation of [^{3}H]thymidine into HSV-infected HEL cells. After 1 h of virus adsorption, the cells were cultured for 4 h with various concentrations of the polyoxometalates and pulse-labeled with [^{3}H]thymidine for 30 min. The cells were then collected, treated with 5% TCA, and precipitated onto glass microfilters. Radioactivity detected in acid-insoluble fractions was identified as viral DNA, as it corresponded to the specific viral DNA density.

Virucidal effect

About 10^7 PFU/ml of the virus were treated with 200 μg/ml of PM-19, incubated for 1 h at 0°C or at 37°C, and then further diluted with HBSS containing 1% BPA. Virus titers were estimated by plaque assay in Vero cells.

Other DNA viruses

The antiviral activity of polyoxometalates against other DNA viruses was determined with the $TCID_{50}$ reduction test. Adenoviruses were tested in Vero cells following an observation period of 7 days. Similarly, HCMV was tested in HEL cells following an observation period of 10 days.

TABLE 1

Activity of polyoxometalates against herpes simplex virus

C:P[a]	General formula[b]	Polyoxometalate	Conc. (μg/ml)	Reduction of $TCID_{50}(-\log_{10})$[c]		MNTD[d] (μg/ml)
				HSV-1	HSV 2	
Heteropolyanions						
1:12	$[X^{n+}M_{12}O_{40}]^{(8-n)-}$					
	Keggin structure	PM-1 $K_5[BW_{12}O_{40}]$	20	<0.5	<0.5	>200
			50	1.0	1.0	
			100	2.2	>3.2	
			200	>3.0	>3.2	
		PM-19 $K_7[Ti_2W_{10}PO_{40}]$[e]	20	1.0	ND[f]	>200
			50	2.0	ND	
			100	>3.0	>3.0	
			200	>3.0	>3.0	
		PM-43 $K_5[SiVW_{11}O_{40}]$	100	2.4	>3.0	>200
			200	>3.0	>3.0	
		PM-44 $K_5[PVW_{11}O_{40}]$	100	2.2	>3.0	>200
			200	>3.0	>3.0	
2:18	$[(X^{n+})_2M_{18}O_{62}]^{(16-2n)-}$					
	Dawson structure	PM-27 $K_6[P_2W_{18}O_{62}]$	20	3.0	ND	50
			50	>3.0		
		PM-28 $K_8[P_2MnW_{17}O_{62}]$	10	2.7	ND	20
			20	>3.0		
Antiherpetic compounds						
	Ara-A		20	<0.5	<0.5	30
			30	1.0	1.0	
	Acyclovir		10	2.7	3.0	>500
			50	>3.0	>3.0	
			100	>3.0	>3.0	
	Phosphonoacetic acid		20	<0.5	1.0	200
			50	2.0	>3.0	
			100	>3.0	>3.0	

[a]C:P represents the ratio between the central and peripheral elements in the polyoxometalates.
[b]General formula: M is usually tungsten or molybdenum and X is one of 4-coordinated elements, such as P(V), Si(III), B(III).
[c]Reduction of $TCID_{50}$ is calculated as $\log_{10}$ $TCID_{50}$ (control) minus $\log_{10}$ $TCID_{50}$ (drug-treated group).
[d]Maximum nontoxic dose: the highest concentration that causes no morphological change of the host cells.
[e]Actually occurs as its hexahydrate.
[f]ND: not determined.

Results

Heteropolyanions are inorganic complexes with molecular weights ranging from about 1000 to 7000. They can form various coordination structures in which the central atom, often termed heteroatom, is surrounded by polyoxoanions. The shape of the heteropolyanions depends upon the ratio between central atom and peripheral anions. We have examined several different heteropolyanionic compounds (Tables 1 and 2) against HSV. Heteropolyanions with the general formula: $[X^{n+}W_{12}O_{40}]^{(8-n)-}$, where X is a 4-coordinated element, such as B(III), Si(III) and P(V), which form the so-called Keggin structure (1:12), were found to inhibit both HSV replication. Antiviral activity was still detected when the central phosphorus atom was replaced by silicon or boron. Furthermore, one or two of the peripheral tungsten ions could be replaced by other transition metals. Complexes of the Dawson type (2:18) also proved active. The Dawson structure can be considered as a condensation of two halves of the Keggin structure from which three WO_6 units are removed and six oxygen atoms are shared. As long as the basic Keggin structure is maintained, the antiviral activity is preserved. Other heteropolyanions with a (1:9) or (1:10) ratio as well as other types did not demonstrate any antiherpetic activity (Table 2).

Among the Keggin-type polyoxometalates, $K_7[Ti_2W_{10}PO_{40}]\cdot 6H_2O$ (PM-19) had the most widely activity range. Thus further antiviral activity studies were

TABLE 2

List of the polyoxometalates tested which were ineffective against herpes simplex virus

C:P[a]	General formula[b]	Polyoxometalates	
Heteropolyanions			
1:9	$[X^{n+}M_9O_{32}]^{(10-n)-}$		Isopolyanions
	Waugh structure	PM-5 $Na_6[NiMo_9O_{32}]$	Metatungstate
		PM-6 $Na_6[MnMo_9O_{32}]$	PM-9 $[NH_3iPr]_{10}[H_2W_{12}O_{42}]$
1:10	$[XW_{10}O_{36}]^{8-}$		Paramolybdate
		PM-7 Na_9 $[EuW_{10}O_{36}]$	PM-8 $[NH_3iPr]_6[Mo_7O_{24}]$
		PM-11 Na_9 $[CeW_{10}O_{36}]$	PM-20 $[NH_4]_6[Mo_7O_{24}]$
		PM-12 Na_9 $[NdW_{10}O_{36}]$	PM-23 $K_6[Mo_7O_{24}]$
Other heteropolyanions			
	PM-10 $K_7[NiV_{13}O_{38}]$		
	PM-13 $K_5NaH[Mo_9V_3O_{38}]$		
	PM-14 $K_4[V_2W_4O_{19}]$		
	PM-15 $Na_6[Mo_6V_2O_{26}]$		
	PM-2 $K_{18}[KSb_9W_{21}O_{86}]$		
	PM-3 $K_{27}[KAs_4W_{40}O_{140}]$		

[a]C:P represents the ratio between the central and peripheral elements in the polyoxometalates.
[b]General formula: M is usually tungsten or molybdenum and X is one of 4-coordinated elements, such as P(V), Si(III), B(III).
[c]$[NH_3iPr]$: Isopropyl ammonium.
Molecular weight of PM-19 ($K_7[Ti_2W_{10}PO_{40}]\cdot 6H_2O$): 2986.

TABLE 3

Inhibition of plaque formation of various strains of HSV type 1 by polyoxometalate PM-19

	Conc. (μg/ml)	Reduction of plaque count (%)[a]				
		KOS	Hayashida	R6′	R8′	Tomioka
PM-19	10	7	19	18	20	
	20	14	30	38	42	17
	50	54	80	89	85	53
	100	100	100	100	100	100
	200	100	100	100	100	100
EC_{50}[b]	PM-19	47	29	25	24	47
	ACV	ND[c]	0.34	54	44	ND

[a]Vero cells were infected with about fifty plaque forming units of each HSV-1 strain. The reductions are presented as percentages compared to the control. Each experiment was performed in triplicate.
[b]50% Plaque-reducing concentration.
[c]ND: not determined.

carried out with PM-19. The toxicity of PM-19 to the host cells was found to be minimal; at 200 μg/ml PM-19 did not affect the growth of Vero cells over a 72-h period, and only 13% inhibition was observed if the concentration of PM-19 was increased to 500 μg/ml.

PM-19 inhibited plaque formation of several HSV strains including acyclovir-resistant strains R6′ and R8′ (Table 3). The 50% plaque-inhibiting concentration (EC_{50}) for the different HSV strains was between 20 and 50 μg/ml. PM-19 also inhibited the single cycle growth of HSV, if the infected cells were treated with various concentrations of the compound following the 1-h virus adsorption period (Table 4). PM-1, $K_5[BW_{12}PO_{40}]\cdot 5H_2O$, was as effective

TABLE 4

Effect of polyoxotungstates and other antiherpetic compounds on a single cycle growth of HSV type 1

Compound	Conc. (μg/ml)	Virus yield			
		Intracellular		Extracellular	
		PFU/dish	Inhibition (−Log T/C)	PFU/dish	Inhibition (−Log T/C)
Control		4.07×10^8	–	3.00×10^7	–
Ara-A	30	7.38×10^4	4.74	3.39×10^3	3.88
IDU	30	1.00×10^6	2.61	7.50×10^2	4.60
Acyclovir	100	8.50×10^2	5.68	$<5.00 \times 10$	>5.78
PM-19	50	6.00×10^5	2.83	5.83×10^5	1.71
	100	1.12×10^5	3.56	2.10×10^5	2.16
	200	1.07×10^3	5.58	4.95×10^4	2.78
PM-1	50	1.34×10^6	2.48	7.13×10^5	1.62
	100	1.99×10^5	3.31	8.38×10^4	2.55
	200	$<2.50 \times 10$	>7.21	$<5.00 \times 10$	>5.78

Vero cells were infected with HSV type 1 (KOS strain) at an MOI of 0.1. The compounds were added to the cell cultures at 1 h after virus inoculation. Samples were collected at 20 h post-infection.

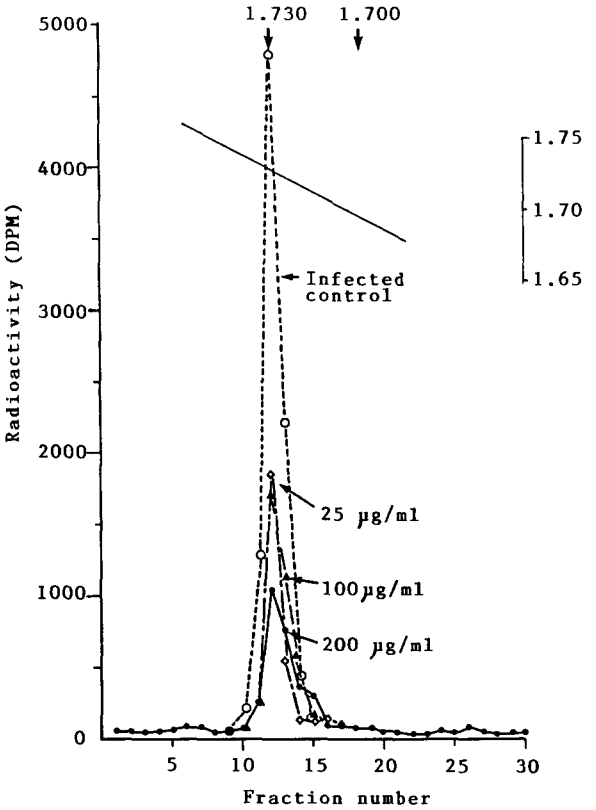

Fig. 1. Equilibrium density sedimentation profile of newly synthesized DNA isolated from virus-infected HEL cells 5 h post-infection. PM-19 was added at 1 h after virus inoculation.

TABLE 5

Effect of PM-19 on the incorporation of [*methyl*-^{3}H]thymidine into the acid-insoluble fraction of HSV-infected HEL cells

Conc. of PM-19 (µg/ml)	HSV-1 (Hayashida)						Non-infected	
	MOI = 0.5		MOI = 2.0		MOI = 5.0			
	CPM	Inhibition (%)	CPM	Inhibition (%)	CPM	Inhibition (%)	CPM	Inhibition (%)
0	393	–	1034	–	1414	–	3310	–
2	244	37.9	694	32.9	1094	22.6	3432	0
5	131	66.7	360	65.2	656	53.8	3761	0
10	95	75.8	251	75.7	359	74.6	3629	0
20	43	89.1	183	82.3	286	79.8	3650	0
50	31	92.1	190	81.7	281	80.2	3891	0
100	44	88.8	163	84.3	244	82.7	4511	0
200	37	90.6	202	80.5	267	81.2	4312	0

HEL cell were infected with HSV type 1 (Hayashida strain) at different multiplicities of infection (MOI). PM-19 was added to the cell cultures at 1 h after virus inoculation. Cells were pulse-labeled with tritiated thymidine between 5–5.5 h post infection. Immediately thereafter, the cells were washed with cold PBS and treated with cold 5% TCA.

effective as PM-19. Thus, from the results presented in Table 4 it appears that Keggin-type heteropolyanions may influence a replicative step subsequent to the virus adsorption step.

Since PM-19 appeared to influence virus replication, its inhibitory effect on the viral DNA synthesis was analyzed by equilibrium CeCl density-gradient centrifugation. Most of the radioactivity detected in the acid-insoluble (DNA) fraction of infected cells was viral-specific DNA (Fig. 1). PM-19 markedly inhibited viral DNA synthesis. Inhibition of viral DNA synthesis was observed from a concentration of 5 μg/ml (Table 5). Inhibition of viral DNA synthesis increased with increasing concentrations of the compound (within the range of 5–50 μg/ml). The radioactivity in the acid-insoluble fraction was identified as viral DNA, since most of the acid-insoluble radioactivity of the infected cells had the specific density of viral DNA.

Pretreatment of the cells with PM-19 from 15 h before infection had no inhibitory effect on the HSV plaque formation; however, the virus plaque count was significantly decreased when PM-19 (25 μg/ml) was present during the virus adsorption period (0–1 h, see Fig. 2). Plaque formation was inhibited by about 80% at a PM-19 concentration of 50 μg/ml, and no further increase in inhibition was noted at higher concentrations of PM-19.

PM-19 and PM-1 also showed significant activity against some other DNA viruses (Table 6). PM-19 was active against HCMV. It only had a weak effect on vaccinia virus and adenoviruses. In contrast, PM-1 was significantly active against vaccinia virus.

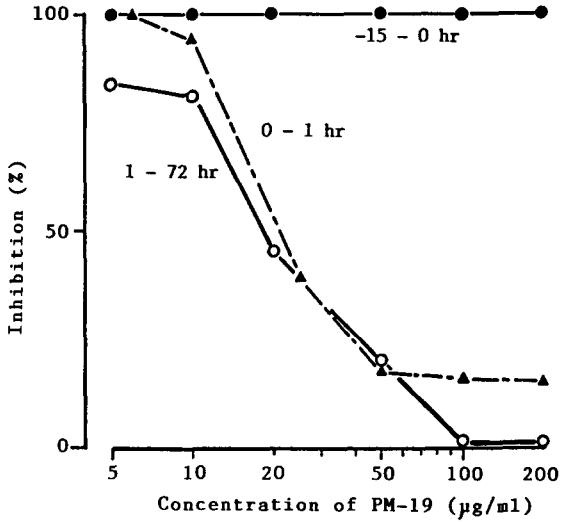

Fig. 2. Effect of PM-19 on HSV plaque formation following drug treatment for different time periods. ●: cells were pretreated with PM-19 for 15 h before infection; ▲: cells were treated with PM-19 during the 1-h virus adsorption period; ○: cells were treated with PM-19 for 72 h starting at 1 h after virus inoculation.

TABLE 6
Effect of PM-19 and PM-1 on viruses other than HSV

	Conc. (μg/ml)	Reduction of $TCID_{50}$ (log_{10})				
		HCMV	VZV	Vaccinia virus	Adenovirus	
					type 1	type 5
PM-19	100	2.0[a]	<0.5	<0.5	<0.5	<0.5
	200	2.2	<0.5	<0.5	<0.5	<0.5
PM-1	100	<0.5	<0.5	2.0	<0.5	<0.5
	200	<0.5	<0.5	2.0	<0.5	<0.5
ACV	100	1.3	3.2	<0.5	<0.5	<0.5
Ara-A	30	2.0	1.0	1.8	<0.5	<0.5
IDU	30	>3.5	1.0	<0.5	<0.5	<0.5

[a]The values represent the differences in $TCID_{50}$ between the control (virus-infected) group and treated (virus-infected) group.

Discussion

This study has revealed a distinct antiherpetic activity for Keggin-type heteropolyoxotungstates. The central atom can be replaced by 4-coordinated elements such as P(V), Si(III) and B(III). Also, the peripheral tungstens can be replaced by other transition metals. Dawson-type structures, in which two Keggin-type polyanions are condensed to make one molecule, were also found to be active, but other polyanion types tested were inactive.

HPA23 and HPA39 (which corresponds to PM-2 in this study), that belong to the heteropolyanion family ($[Me^{n+}Sb_9W_{21}O_{86}]^{(19-n)-}$, Me being an alkaline earth cation) have been reported to exhibit a strong antiviral activity against various RNA and DNA viruses, specifically retroviruses (Jasmin et al., 1974; Werner et al., 1976; Souyri-Caporale et al., 1984; Larnicol et al., 1981). Their mode of action has been extensively studied, and they were found to inhibit DNA and RNA polymerases from various microorganisms and mammals by interacting with the DNA/template (Ono et al., 1984,1988; Schinazi et al., 1989; Wondrak et al., 1988). They also interfere with virus adsorption, and their structure can be described as an association of two triantimonate(III) $Sb_3O_7^{5-}$ and three 7-tungsto-antimonate(III) $SbW_7O_{28}^{11-}$. In their center HPA23 and HPA39 possess Na^+ and K^+, respectively (Fisher et al., 1976; Michelon et al., 1980). HPA44, consisting of the ion $[MeAs_4W_{40}O_{140}]^{27-}$, has also been reported to inhibit rabies virus in vitro and in vivo (Bussereau et al., 1983,1988). PM-3 belongs to this ion family.

Hill et al. (1990) reported that only compounds containing more than six metal atoms show antiviral activity, and that no close correlation exists between the molecular size, charge, or charge density of the polyoxometalates

and their anti-HIV activity. From the present results it appears, however, that the Keggin-type structure is closely associated with antiherpetic activity. All Keggin-type and Dawson-type structures tested were active, although they differed in antiviral activity as well as cytotoxicity. PM-19 was found to have the widest range of antiviral activity and was also the least toxic of the Keggin complexes.

To some extent antiviral activity of PM-19 may be attributed to the stability of the compound, as PM-19 has a greater surface negative charge than PM-1 or PM-43. An increased negative charge results in an increased resistance to nucleophilic degradation. Thus PM-19 may be more stable than other polyoxometalates in culture fluid or cells.

The strongly negative charge of PM-19 may also be important for antiviral activity. Initially, HSV has to bind to heparan sulfate on the cellular surface (WuDunn et al., 1989), and the basic-FGF (fibroblast growth factor) receptor is thought to be the cellular entry portal of HSV (Kaner et al., 1990). PM-19 may thus inhibit viral binding to the cell surface receptors because of the strong negative charge on the heteropolyanion's surface. Inhibitory activity against virus adsorption seems to be a common characteristic of polyanions (Vaheri, 1964; De Clercq, 1986,1990; Mitsuya et al., 1988).

PM-19 has also been found to inhibit HIV-1 replication, by interfering with an early step, before virus penetration into the target cells (Inoue et al., 1990; Take et al., 1991). However, virus adsorption may not be the sole or main target for the antiviral effect of PM-19 against HSV type 1, since at 12.5 μg/ml PM-19 was not found to interfere with virus adsorption, although viral DNA synthesis was significantly inhibited at a concentration of 5 μg/ml. Furthermore, the single-cycle growth of HSV was markedly inhibited if infected cells were treated with PM-19 following virus adsorption to the cells.

It is conceivable that PM-19 not only interferes with virus adsorption, but also a subsequent step in the virus replicative cycle, thus leading to an inhibition of viral DNA synthesis. The virus-specific event (other than virus adsorption) with which PM-19 interferes remains to be determined. Finally, PM-19 was also found to be effective in vivo: it significantly increased the mean survival time of HSV-1-infected mice which had been previously immunocompromised (Fukushima K, personal communication). PM-19 may thus have therapeutic potential as an antiherpetic agent.

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