INHIBITION OF REPLICATION OF HUMAN IMMUNODEFICIENCY VIRUS BY A HETEROPOLYOXOTUNGSTATE (PM-19)

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A Keggin polyoxotungstate PM-19 $K_7[PTi_2W_{10}O_{40}]\cdot 6H_2O$ was found to be a potent inhibitor of the replication of human immunodeficiency virus (HIV), which causes acquired immunodeficiency syndrome (AIDS), in OKT4$^+$ cells. In contrast, the effect of HPA 23 $(NH_4)_{17}Na[NaSb_9W_{21}O_{86}]$, an inhibitor of reverse transcriptase of HIV, was not significant.

KEYWORDS heteropolyoxotungstate; human immunodeficiency virus (HIV); acquired immunodeficiency syndrome (AIDS); antiviral agent; Keggin structure

Some of the heteropolyoxotungstates, $[SiW_{12}O_{40}]^{4-}$, $[BW_{12}O_{40}]^{5-}$, $[P_2W_{18}O_{62}]^{6-}$, $[As_2W_{18}O_{62}]^{6-}$ and $[Sb_9W_{21}O_{86}]^{19-}$ are potent inhibitors of cellular, bacterial and viral DNA and RNA polymerases and have antiviral effects both *in vitro* and *in vivo* at non-toxic doses.[1-11]

Initiallly described as ammonium 5-tungsto-2-antimoniate, later studies indicated that HPA 23 is a mineral-condensed heteropolyanion (HPA) with the formula ammonium 21-tungsto-9-antimoniate $(NH_4)_{17}Na[NaSb_9W_{21}O_{86}]$.[6] It inhibited mouse leukemia-sarcoma virus *in vitro*, and reduced the development of disease caused by Friend leukemia or Moloney murine sarcoma virus.[3] HPA 23 is a competitive inhibitor of reverse transcriptases of murine and human retroviruses with respect to template/primer.[10,11] Furthermore, the reverse transcriptase activity of human immunodeficiency virus (HIV), a causative agent of acquired immunodeficiency syndrome (AIDS), is completely inhibited by HPA 23 at a concentration of 60 μg/ml.[10] However, the drug has little effect on the replication of HIV *in vitro*.[12]

Recently we observed antitumor activity in certain polyoxomolybdates, for example $(NH_3Pr^i)_6[Mo_7O_{24}]\cdot 3H_2O$ (PM-8),[13,14] and marked inhibition of the replication of Herpes simplex virus (HSV) by some Keggin polyoxotungstates such as $K_7[PTi_2W_{10}O_{40}]\cdot 6H_2O$ (PM-19).[15] PM-19 is active against a broad spectrum of DNA viruses *in vitro*, and Herpes simplex virus type 1 (HSV-1) *in vivo*.

In our screening of a series of heteropolyoxometalates for inhibitors of the replication of HIV *in vitro*, PM-19 was the most potent one. In this paper, the effect of PM-19 on the replication of HIV is described in comparison with that of HPA 23. To our knowledge, this is the first report of the inhibition of HIV replication *in vitro* by a polyoxometalate.

PM-19 and HPA 23 were prepared as reported previously.[2,14] The replication of HIV was assayed by the previous method[16] which was based on the bio-assay system established by Harada *et al.* using HTLV-I-carrying MT-4 cells.[17] Briefly, HTLV-III$_B$ one of the HIV strains was propagated in MT-4 cells in the absence or presence of various concentrations of test compounds. Four days after the viral infection, the number of viable cells was counted by a trypan blue dye exclusion test and the expression of viral specific antigens was assayed by an indirect immuno-fluorescence (IF) technique.

Figure 1 illustrates the ability of PM-19 to maintain the survival of MT-4 cells exposed to HIV at concentrations higher than 3.12 μg/ml. There was no toxicity up to a concentration of 200 μg/ml. In contrast, HPA 23 showed only a marginal effect on the survival of HIV-infected MT-4 cells at concentrations

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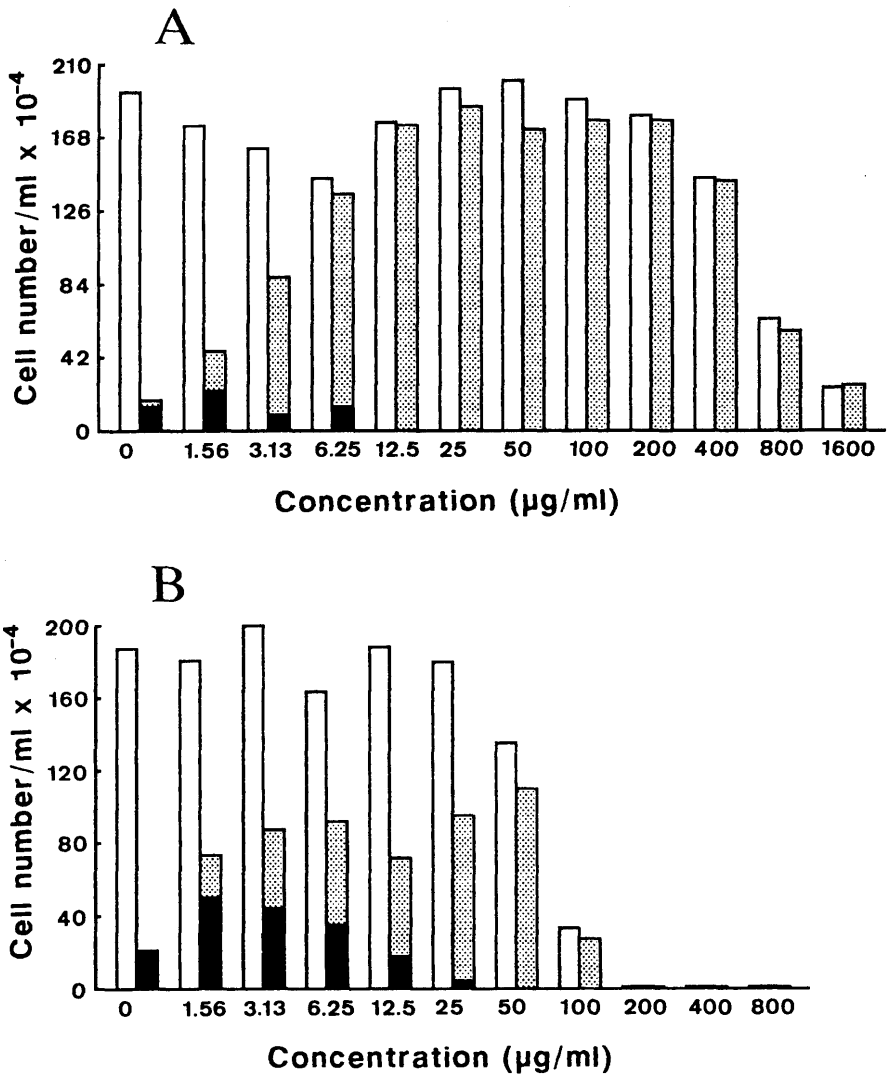

Fig. 1. Inhibition of Cytopathic Effect of HIV on MT-4 Cells and Replication of HIV in MT-4 Cells

MT-4 cells and HIV infected MT-4 cells were cultured in the absence or presence of various concentrations of PM-19 (A) or HPA 23 (B). The cells were infected with HIV at a multiplicity of infection of 0.02 and incubated for 1 hour. After adsorption, drugs were added to the culture and the cell number was adjusted at 2 x 10^5 cells/ml.

The viable cells were counted by a trypan blue dye exclusion method 4 days after the viral infection. The number of IF-positive cells in HIV infected MT-4 cells was determined by an indirect immuno-fluorescence method. □, control culture of MT-4 cells without HIV infection; ▨, HIV infected MT-4 cells; ■, IF-positive cells in HIV infected MT-4 cells.

partially toxic to MT-4 cells. The results for HPA 23 is in good agreement with those of Balzarini *et al.*[12] The PM-19 inhibition of HIV replication was further confirmed by the expression of virus-specific antigens. No IF-positive cells were detected in a population of MT-4 cells infected with HIV in the presence of 12.5 μg/ml or more PM-19, while the cytopathic effect of HIV against MT-4 cells was completely suppressed by equivalent concentrations of PM-19.

Both PM-19 and HPA 23 belong to a group of mineral-condensed heteropolyanions. However, PM-19 is distinguishable from HPA 23 by its inhibition of HIV replication *in vitro*. Polyanionic substances like heparin, pyran or dextran sulfate modify the cell membrane and affect the adsorption and penetration of viruses.[18,19] The inhibition of reverse transcriptase in retroviruses is another possible effect on viral replication. In fact, PM-19 inhibited avian myeloblastosis virus (AMV) reverse transcriptase with an ID_{50} of approximately 10 μg/ml (data not shown). Under the same experimental conditions, HPA 23

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inhibited AMV reverse transcriptase activity by 50% at a concentration of 18 μg/ml. Thus, PM-19 shares many biological and physicochemical properties with HPA 23, except that HIV replication *in vitro* is suppressed by PM-19 but not by HPA 23.

The selective susceptibity of the replication of HIV *in vitro* to some heteropolyoxometalates such as PM-19 (the structure-activity relationship among heteropolyoxometalates) and the mode of action by which PM-19 interferes with HIV replication remain to be elucidated.

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