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

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A Keggin polyoxotungstate PM-19 $K_2\{\text{PTi}_{12}\text{W}_{18}\text{O}_{60}\}\cdot6\text{H}_2\text{O}$ 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$_4$)$_2$Na[NaSb$_5$W$_2$O$_{26}$], 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$_{40}$]$^{8-}$, [BW$_{12}$O$_{40}$]$^{8-}$, [P$_2$W$_{16}$O$_{62}$]$^{6-}$, [As$_2$W$_{16}$O$_{62}$]$^{6-}$ and [Sb$_5$W$_2$O$_{26}$]$^{18-}$ 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.\(^{1-11}\)

Initially 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$_4$)$_2$Na[NaSb$_5$W$_2$O$_{26}$].\(^{12}\) It inhibited mouse leukemia-sarcoma virus in vitro, and reduced the development of disease caused by Friend leukemia or Moloney murine sarcoma virus.\(^{13}\) 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 \(\mu\text{g}/\text{ml}\).\(^{10}\) 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$_2$Pr$^+$)$_2$[Mo$_7$O$_{24}$]$\cdot3$H$_2$O (PM-8),\(^{13,14}\) and marked inhibition of the replication of Herpes simplex virus (HSV) by some Keggin polyoxotungstates such as $K_2\{\text{PTi}_{12}\text{W}_{18}\text{O}_{60}\}\cdot6\text{H}_2\text{O}$ (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.\(^{9,14}\) The replication of HIV was assayed by the previous method\(^{14}\), which was based on the bio-assay system established by Harada \etal using HTLV-I-carrying MT-4 cells.\(^{17}\) Briefly, HTLV-IIIb 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 \(\mu\text{g}/\text{ml}\). There was no toxicity up to a concentration of 200 \(\mu\text{g}/\text{ml}\). In contrast, HPA 23 showed only a marginal effect on the survival of HIV-infected MT-4 cells at concentrations...
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 immunofluorescence 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. 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. 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
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 susceptibility 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.

ACKNOWLEDGMENT This work was supported in part by Grant-in-Aid for General Scientific Research from the Ministry of Education, Science and Culture, Japan.

REFERENCES

(Received November 20, 1989)