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Preferential Inhibition of Herpes-Group Viruses by Phosphonoacetic Acid: Effect on Virus DNA Synthesis and Virus-Induced DNA Polymerase Activity

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In tissue culture phosphonoacetic acid (PAA) specifically inhibited DNA synthesis of human cytomegalovirus (CMV), murine CMV, simian CMV, Epstein-Barr virus, and *Herpesvirus saimiri*. Fifty to one hundred micrograms per milliliter PAA completely inhibited viral DNA synthesis with no significant damage to host cell DNA synthesis. *In vitro* DNA polymerization assays showed that 10 μg/ml of PAA specifically inhibited partially purified human CMV-induced DNA polymerase, while little inhibition of host-cell DNA polymerase activity was found. The specific inhibition of herpes-group virus DNA synthesis with little toxicity to host cells suggests that PAA has great potential as an antiherpesvirus therapeutic agent.

Phosphonoacetic acid (PAA) has been shown to be a very active compound against herpes simplex dermititis in mice and herpes simplex keratitis in rabbits (11). In tissue culture replication of herpes simplex virus and viral DNA are impeded by PAA while host-cell function remains unharmed. In a study with partially purified herpes simplex virus-induced DNA polymerase, Mao *et al.* (7) demonstrated that the inhibition involved direct interaction between the compound and the virus-induced DNA polymerase.

We report here similar observations. PAA preferentially inhibited the replication of DNA of human CMV, murine CMV, simian CMV, Epstein-Barr virus, and *H. saimiri*. Also, DNA polymerases isolated from human CMV-infected human fibroblast (WI-38) were partially purified, and the effect of PAA on the activity of these enzymes was studied *in vitro*.

Figure 1 shows that PAA inhibits human CMV DNA synthesis in virus-infected WI-38 cells. These cells in Falcon flask (75 cm^2, No. 3024) were infected with human CMV (strain AD-169) at the multiplicity of 1-2 PFU. After virus absorption, PAA was added at various concentrations. At various intervals after infection the virus-infected or mock-infected cells were lysed with 1% sodium dodecyl sulfate buffer (1% sodium lauryl sulfate, 0.01 *M* Tris-HCl, pH 7.8, and 0.01 *M* EDTA) in order to quantitate viral DNA. The total DNA was extracted, and hybridization with CMV-specific [^{3}H] cRNA was carried out as previously described (4). Human CMV DNA synthesis was completely inhibited in the presence of 50 μg/ml of PAA. The minimum dose necessary for inhibition was between 20 and 50 μg/ml.

The effect of PAA on host-cell DNA synthesis (Fig. 2) was measured as previously described (3) by the incorporation of [^{3}H]thymidine into acid-insoluble material from mock-infected cells treated with PAA before confluent monolayers had formed. Partial inhibition of WI-38 DNA synthesis was observed with concentrations of PAA between 100 and 1000 μg/ml. At a concentration of 100 μg/ml only 15% of host DNA synthesis was inhibited; whereas viral DNA synthesis was completely inhibited

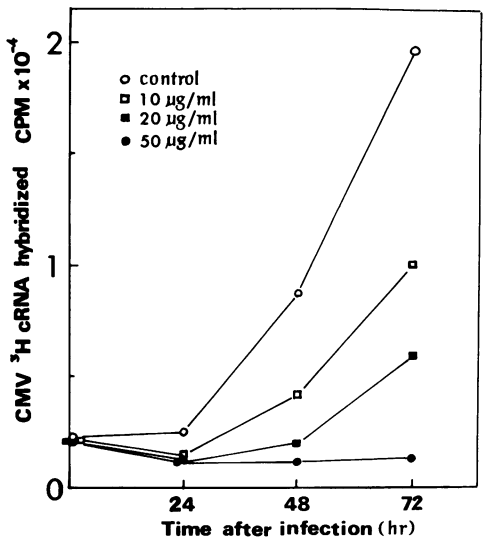

FIG. 1. Effect of PAA on human CMV DNA synthesis. PAA at a final concentration of 0 to 50 μg/ml was added to human CMV-infected cell cultures after virus absorption. At various times after infection, the DNA was extracted from the cell cultures for virus genome quantitation by ^{3}H-labeled cRNA–DNA membrane hybridization. Fifty micrograms of DNA and 1.2 × 10^5 cpm CMV-specific [^{3}H] cRNA (sp act 1 × 10^7 cpm/μg) were applied to each filter. The nucleic acid hybridization was carried out as described previously (4).

at this dose. Even at the highest dose of PAA tested, 1000 μg/ml, cell DNA synthesis was 30% of DNA synthesis in untreated cultures. No obvious morphological alteration was observed in confluent cells in the presence of up to 1000 μg/ml of PAA. This compound has less effect on the confluent cells than on cells in the log phase of growth.

The effect of PAA on partially purified host cell and virus-induced DNA polymerase activity is shown in Fig. 3. Two large molecular weight host-cell DNA

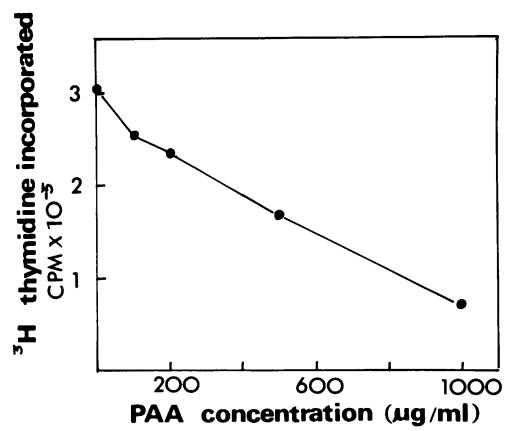

FIG. 2. Influence of PAA on the DNA synthesis of WI-38 human fibroblast. PAA was added to WI-38 cells in Falcon Petri dishes 47 hr after subcultures, when the cells reached 50 to 60% confluency. The cells were then labeled with 3 μCi/ml of [^{3}H]thymidine (Nuclear Dynamics, sp act 45 Ci/m*M*) for 46 hr. The cell cultures were then lysed with SDS buffer, collected, and precipitated with ice-cold trichloroacetic acid. The acid-insoluble material was then collected by membrane filters and radioactivity was measured (3).

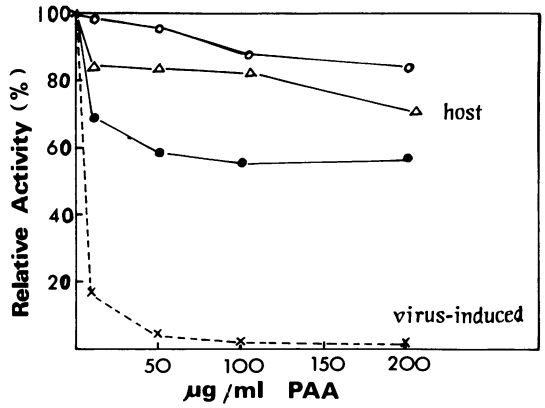

FIG. 3. Effect of PAA on partially purified host-cell and virus-induced DNA polymerase activity. The method for assaying DNA polymerase activity was described in a previous publication (2). PAA was added to the reaction at a final concentration of up to 200 μg/ml. The polymerization obtained without PAA was used as 100% activity. Symbols: (○) Large molecular weight host-cell DNA polymerase (DNA polymerase α) isolated from nuclei fraction (NuD2P1. See Ref. 2); (●) large molecular weight host-cell DNA polymerase isolated from cytoplasmic fraction (CyD2P2, see Ref 2); (△) small molecular weight enzyme isolated from cytoplasmic fraction (DNA polymerase β, CyD1, see Ref. 2); (x) virus-induced DNA polymerase.

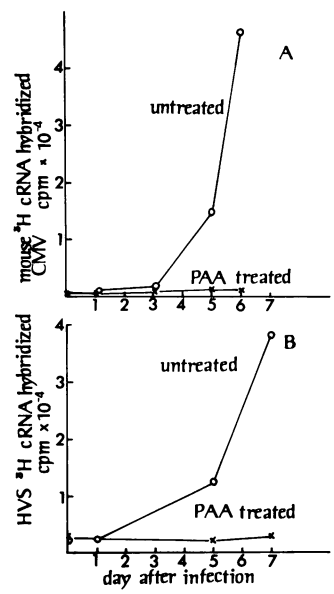

FIG. 4. Effect of PAA on murine CMV and *Herpesvirus saimiri* DNA Synthesis. Mouse embryo fibroblast cultures and owl-monkey kidney cell cultures were infected with murine CMV (Smith strain, Ref. 6) and *H. Saimiri*, respectively. PAA at a concentration of 100 μg/ml was added to the infected cultures immediately after virus absorption. Viral DNA syntheses were detected by cRNA–DNA hybridization using viral-specific [³H] cRNAs as probes. Specific activity for [³H] murine CMV cRNA and [³H] HVS cRNA were both 1×10^7 cpm/μg. Panel A shows the effect of PAA on mouse CMV DNA synthesis. Panel B shows that in *H. saimiri*.

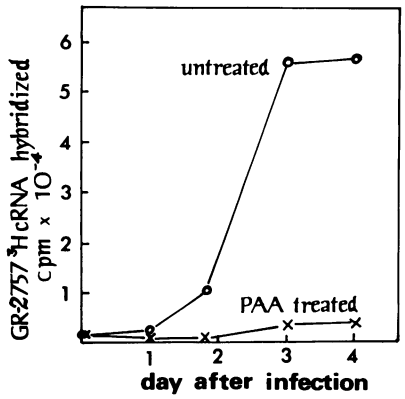

FIG. 5. Effect of PAA on simian CMV (GR 2757) DNA synthesis in virus-infected WI-38 cells. Simian CMV (strain GR 2757, Ref. 1) was used for this study. PAA at a concentration of 100 μg/ml was used. The viral DNA content was also monitored by [^{3}H]cRNA-DNA membrane hybridization as described before (4) and in Fig. 1. The specific activity of [^{3}H]simian CMV cRNA was about 1×10^7 cpm/μg.

polymerases (DNA polymerase α; NuD2P1, and CyD2P2, see Ref. 2), one small molecular weight host-cell polymerase (DNA polymerase β, NuD1, Ref. 2), and human CMV-induced DNA polymerase (2) were used in this experiment. A remarkably preferential inhibition of virus-induced DNA polymerase was observed when 10 μg/ml PAA was added to the reaction mixture; more complete inhibition was obtained when the PAA concentration reached the level of 50 μg/ml. However, host-cell enzymes were relatively resistant to PAA, even at a concentration of 200 μg/ml. Evidently the selective inhibition of virus-induced DNA polymerase by PAA accounts for the inhibition of viral DNA synthesis in PAA treated virus-infected cells. Also, PAA appears to interact directly with the virus-induced DNA polymerase and not with the DNA (3).

The study of the effect of PAA on virus DNA synthesis was also extended to non-human CMVs (mouse and simian), cultured in mouse embryo cells and WI-38 cells, respectively, and *H. saimiri* cultured in owl-monkey kidney cells. In all of these cases PAA also inhibited viral DNA synthesis almost totally (Figs. 4 and 5). Mouse embryo cells and owl-monkey kidney cells are more resistant to PAA than WI-38 cells. In the presence of 1000 μg/ml of PAA, the mouse embryo and owl-monkey kidney cell DNA synthesis was still 38 to 42% of that in untreated cultures. *H. saimiri,* isolated from squirrel monkey (8), is highly oncogenic. It invariably causes lethal lymphatic leukemia or malignant lymphoma in nonhuman primates (8).

Finally, the effect of PAA on Epstein–Barr virus (EBV) was studied using the HR1K cell line, an EBV virus-producing Burkitt's lymphoblastoid cell line. The drug was added to the suspension culture at 100 μg/ml. During the experimental period, the cells were subcultured twice a week in RPMI with 15% fetal calf serum and 100 μg/ml of PAA. As shown in Fig. 6, in 1 week the EBV DNA content was dramatically reduced from 450 genome equivalents per cell to 27 genomes, and continued to decline to 4.5 genomes after 4 weeks of treatment, while the EBV DNA content remained constant in untreated cells. The virus capsid antigen (VCA) also decreased proportionally in PAA-treated cells. The growth rate of HR1K was somewhat impeded by PAA. After 4 days in the presence of 100 μg of PAA, the doubling time was increased from 32 hr to 48 hr, and after 8 days it was increased to 96 hr. Among three DNA polymerases isolated from HR1K cells, the low molecular

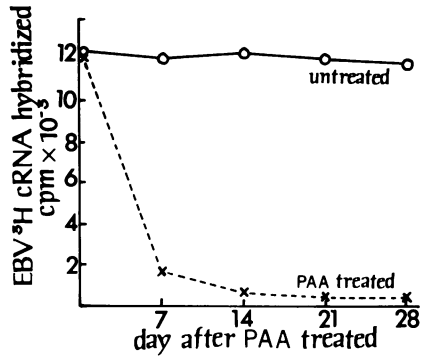

FIG. 6. Effect of PAA on EBV DNA synthesis in HR1K lymphoblastoid cells. PAA was added to EBV suspension cultures at a final concentration of 100 μg/ml. The cell line was subcultured twice a week with RPMI 1640 medium supplemented with 15% fetal calf serum with 100 μg/ml of PAA. Portions of the culture were removed at various times after the drug was added for EBV genome quantitation by viral-specific [^{3}H] cRNA–DNA membrane hybridization as described before (4) and in Fig. 1. The specific activity of [^{3}H]EBV cRNA was about 1×10^7 cpm/μg.

weight enzyme (DNA polymerase β) was resistant to PAA, while the large molecular weight DNA polymerase α and polymerase γ were sensitive to PAA. Fifty micrograms per milliliter of PAA inhibited more than 90% of the activity of polymerases α and γ and *in vitro* assay (manuscript in preparation).

We have also observed the effect of PAA on EBV DNA synthesis in nonproducing Raji lymphoblastoid line, which contains 60 EBV genome equivalents per cell (9). There was no reduction in the number of EBV genome equivalents per cell in Raji cells treated with 100 μg/ml of PAA. This indicated that the mechanism of EBV DNA replication in nonproducing Raji cells might be different from that in producing HR1K cells. More experiments are in progress.

The specific inhibition of herpes-group virus DNA synthesis with no or little toxicity to host cells suggests the PAA has great potential as an anti-herpes-group virus therapeutic agent.

ACKNOWLEDGMENTS

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