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Inhibition of Herpes Simplex Virus Multiplication by the Pokeweed Antiviral Protein

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The pokeweed antiviral protein inhibited the multiplication of herpes simplex virus type 1 in cell culture. The extent of antiviral activity was proportional to the length of time that the antiviral protein was present postinfection. The results demonstrate that the continued presence of the pokeweed antiviral protein is necessary for the maximum inhibition of virus yields.

The pokeweed (*Phytolacca americana*) antiviral protein (PAP) is a basic protein of molecular weight 29,000 (2a) which has been shown to inhibit the transmission of several ribonucleic acid-containing plant viruses (4, 6) as well as the multiplication of poliovirus and influenza virus in cell culture (4, 5). PAP has also been reported to inhibit eucaryotic protein synthesis in cell-free systems derived from rabbit reticulocytes (3) and poliovirus-infected HeLa cells (5). In contrast, PAP was found to have no effect on protein synthesis in uninfected HeLa cells (5), which suggests that PAP is unable to enter intact, uninfected cells. Although the mechanism of PAP's antiviral activity is not known, it has been suggested that the virus-cell interaction may facilitate the entry of PAP into the host cell, followed by PAP's inactivation of the host cell's ribosomes (5). This model implies that PAP may be a general antiviral agent, although PAP's effect has not been tested on deoxyribonucleic acid-containing viruses. The results presented here demonstrate that PAP is a potent inhibitor of herpes simplex virus (HSV) multiplication and suggest that PAP is a general antiviral agent rather than a selective one.

Monkey kidney (Vero) and HeLa cells (Flow Laboratories, Rockville, Md.) were grown as monolayers in Eagle modified minimum essential medium (Earle salts) supplemented with fetal calf serum (10%), glutamine (2 mM), penicillin (50 U/ml), and streptomycin (50 μg/ml). HSV type 1 strain KOS (obtained from P. Schaffer, Harvard Medical School, Boston, Mass.) was propagated in Vero cells, stored at −80°C, and assayed in Vero cells by a plaque method utilizing a 2% methyl cellulose overlay (1). PAP was purified from *P. americana* leaves as previously described (2).

The yield of infectious HSV produced in the presence of varying concentrations of PAP at 37°C is shown in Fig. 1. Inhibition of HSV multiplication by PAP was nearly identical when the virus was propagated in either HeLa or Vero cells. The greatest rate of inhibition was observed as the concentration of PAP increased from 0.1 to 3.0 μM. The concentration of PAP that resulted in the 50% inhibition of HSV multiplication (0.3 μM) was similar to that reported to produce 50% inhibition of poliovirus multiplication in HeLa cells (5). These results demonstrate that PAP exhibits antiviral activity against HSV and suggest that PAP may be an effective inhibitor of deoxyribonucleic acid-containing viruses as well as ribonucleic acid-containing viruses.

To determine whether the antiviral action of PAP was dependent upon its continued presence during the HSV infection cycle, PAP was added to cells simultaneously with virus and removed at various times postinfection (p.i.) by washing the infected cells with Earle balanced salt solution (pH 7.0). The effect of the removal of PAP on the yield of infectious virus is shown in Table 1. The data indicate that the maximum inhibition of virus yields was obtained in the continued presence of PAP. However, partial inhibition of virus yields was obtained if PAP was removed as early as 1 h p.i. Thus the extent of antiviral activity was proportional to the length of time that PAP was present p.i. The results suggest that PAP slowly enters infected cells and that the inhibitory effect of PAP on HSV multiplication is cumulative.

In a similar experiment, PAP was added after virus adsorption (1 h p.i.) and left for the remainder of the infection cycle to determine whether the virus-cell interaction was essential for PAP's antiviral activity (Table 1). The data indicated that PAP was more effective in the inhibition of virus yields when it was present during virus adsorption. The results suggest that some phase of viral adsorption, penetration, or both may enhance the entry of PAP into the infected cell.

The present study demonstrates that PAP is an effective inhibitor of HSV multiplication. We believe that PAP may have some clinical signif-

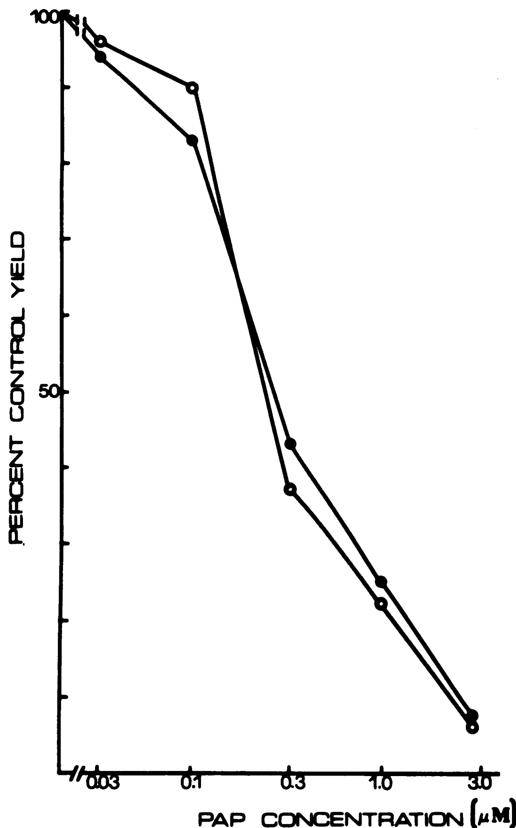

FIG. 1. *Inhibition of HSV multiplication by PAP. Monolayers of either HeLa (○) or Vero (●) cells at 1×10^5 to 2×10^5 cells per plate were infected with HSV at a multiplicity of 5 to 10 plaque-forming units per cell in the presence of the indicated concentrations of PAP. Infected cells were incubated at 37°C for 24 h and virus assays were performed in Vero cells at 34°C after three cycles of freezing and thawing. Results are presented as percent virus yield from untreated, control infected cells; 100% is equal to 2×10^8 to 3×10^8 and 2×10^7 to 3×10^7 plaque-forming units per ml in Vero and HeLa cells, respectively.*

icance as a wide-spectrum antiviral agent because it is a natural product, is readily purified, and has been reported to produce no cytotoxic effects (4, 5). We have also observed that PAP exhibits no cytotoxic effects upon HeLa or Vero cells at the concentrations and times employed in this study. However, cytotoxic effects have been observed upon prolonged incubation (36 h or longer) of PAP with both HeLa and Vero cells (G. M. Aron and J. D. Irvin, manuscript in preparation).

TABLE 1. *Time dependence for inhibition of HSV multiplication by PAP*

Time PAP present p.i. (h)	Percent virus yield[a]	
	Vero	HeLa
No. PAP	(100)	(100)
0–1	77	65
0–3	67	54
0–6	60	51
0–24	30	38
1–24	70	68

[a] PAP (1 μM) was added to Vero and HeLa cell monolayers at 1×10^5 to 2×10^5 cells per plate either simultaneously with virus or 1 h p.i.; it was then removed by washing the cells with Earle balanced salt solution at the indicated times. Cells were infected at a multiplicity of 5 to 10 plaque-forming units per cell. Virus yields were determined at 24 h p.i. after three cycles of freezing and thawing. Results are presented as percent of virus yield from untreated, control infected cells; 100% is equal to 2×10^8 to 3×10^8 and 1×10^7 to 2×10^7 plaque-forming units per ml in Vero and HeLa cells, respectively.

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