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Effect of aphidicolin on DNA synthesis in HSV-1 infected and uninfected Vero cells

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The effect of aphidicolin on DNA synthesis in herpes simplex virus type 1 (HSV-1) infected and uninfected Vero cells was determined by isodensity banding of [^{32}P]-labelled DNA. A 50% inhibition of HSV-1 DNA synthesis was observed at 0.07 μM aphidicolin while 2.1 and 1.3 μM were required to inhibit the cellular DNA synthesis to 50% in infected and uninfected Vero cells, respectively. When the viral DNA synthesis was totally inhibited by 10 μM aphidicolin, the cellular DNA synthesis was inhibited to about 90% in both infected and uninfected cells. Aphidicolin inhibited the cellular DNA synthesis in HSV-1 infected and uninfected Vero cells remaining in the presence of 250 μM foscarnet to the same extent as the DNA synthesis in the absence of foscarnet.

aphidicolin; HSV-1 DNA synthesis; cellular DNA synthesis; foscarnet

Introduction

A selective antiviral compound should inhibit virus multiplication at concentrations not inhibitory to uninfected cells. One method to determine this selectivity, and also, to some extent, the mode of action of antiherpes compounds, is by isodensity separation of viral and cellular DNA, labelled by ortho[^{32}P]phosphate or [^{3}H]thymidine [1–3]. The use of [^{3}H]thymidine incorporation as a measure of DNA synthesis could be misleading if the tested compound interferes with the phosphorylation of thymidine or competes at the incorporation into DNA. To avoid this possibility we have used ortho[^{32}P]phosphate incorporation to measure the effect on DNA synthesis of antiherpes compounds [2,3].

The inhibition of herpes simplex virus type 1 (HSV-1) DNA synthesis by aphidicolin has been reported [4], but the selectivity of inhibition has not been described. The experiments reported here were designed to determine the selectivity of inhibition of herpesvirus DNA synthesis by aphidicolin in infected cells. We also wanted to determine if the cellular DNA synthesis remaining after treating HSV-1 infected and

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uninfected cells with foscarnet was sensitive to aphidicolin and thus due to DNA polymerase α [4–7].

Materials and methods

Chemicals

Foscarnet was synthesized in the Research and Development Laboratories at Astra Läkemedel AB by the methods of Nylén [8], and aphidicolin was kindly supplied by Dr. A.H. Todd, ICI, U.K. Ortho[^{32}P]phosphate (carrier-free, 1 mCi/ml) was supplied by The Radiochemical Centre, Amersham, U.K. and Econofluor scintillation solution, from New England Nuclear, Boston, MA, U.S.A. Pronase (nuclease-free) from Calbiochem, San Diego, CA, and optical grade of CsCl, from Sigma, St. Louis, MO, U.S.A. All other chemicals were of analytical grade.

Cells and virus

Vero cells and HSV-1 strain C42 were used as described previously [2].

Determination of HSV-1 and cellular DNA synthesis

Vero cells grown to about 80% confluence in 35-mm cluster dishes were infected with 2 plaque-forming units of HSV-1 (strain C42) per cell. The test compounds were

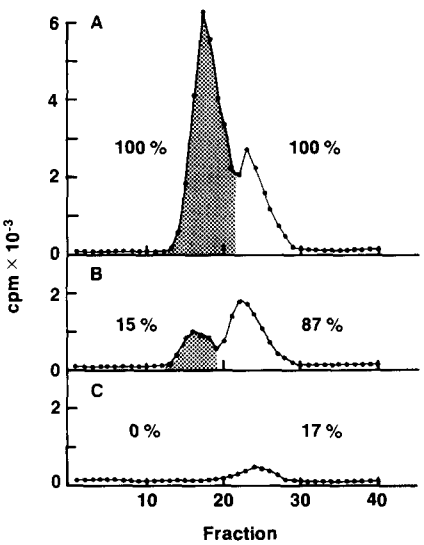

Fig. 1. Effect of aphidicolin on DNA synthesis in HSV-1 infected Vero cells. Viral and cellular DNA were labelled with ortho[^{32}P]phosphate and separated as described in Materials and Methods. The shaded area denotes viral DNA. [^{3}H]Thymidine-labelled DNA from HSV-1 infected and untreated cells was used as internal density marker (not shown). A. Control. B. 1 μM aphidicolin. C. 10 μM aphidicolin.

added to the medium 1 h post-inoculation, and 10 μCi/ml ortho[^{32}P]phosphate was added 3 h post-inoculation. The cells were harvested after 16 h of incubation, and cellular and viral DNA were separated in CsCl gradient as described previously [2]. The density of HSV-1 DNA was determined to 1.726 g/cm^3 and for cellular DNA to 1.710 g/cm^3. [^{3}H]Thymidine-labelled DNA from infected or uninfected untreated cells was used as an internal density marker. Every 50% inhibition value (ID_{50}) was determined at least twice. The mean ID_{50} value reported was based on dose–response curves of aphidicolin (concentration ranging from 0.05 to 10 μM).

Results

Effect of aphidicolin on DNA synthesis in HSV-1 infected Vero cells

The effect of aphidicolin on DNA synthesis in HSV-1 infected Vero cells is shown in Fig. 1 and Table 1. At 0.07 μM aphidicolin HSV-1 DNA synthesis was inhibited to 50% while 2.1 μM was required to inhibit cellular DNA synthesis to the same extent (Table 1). When the viral DNA synthesis was totally inhibited by 10 μM aphidicolin, the cellular DNA synthesis was inhibited to about 90% (Fig. 1 and Table 2).

Effect of aphidicolin on DNA synthesis in uninfected Vero cells

The cellular DNA synthesis in uninfected cells was inhibited to the same extent as in the HSV-1 infected cells by aphidicolin. At 1.3 μM aphidicolin DNA synthesis was inhibited to 50% (Table 1) and at 10 μM to about 90% (Fig. 2 and Table 2).

Effects of foscarnet and aphidicolin on cellular DNA synthesis

At 250 μM foscarnet, HSV-1 DNA synthesis was inhibited to 85% while the cellular DNA synthesis was only slightly affected, both in infected and uninfected cells (Table 2). On the other hand, a combined treatment with 250 μM foscarnet and 10 μM aphidicolin, the cellular DNA synthesis in infected and uninfected cells was inhibited to about 90% (Table 2).

TABLE 1

Inhibition of HSV-1 and cellular DNA synthesis by aphidicolin

Cells	Concentrations giving 50% inhibition (μM)[a]
HSV-1 infected cells	
HSV-1 DNA	0.07
Cell DNA	2.1
Uninfected cells	
Cell DNA	1.3

[a] The ID_{50} values from the individual experiments ranged from 0.05 to 0.09 μM for HSV-1 DNA, from 1.4 to 2.7 μM for cell DNA in the infected cells and from 0.4 to 2.2 μM for all DNA in the uninfected cells.

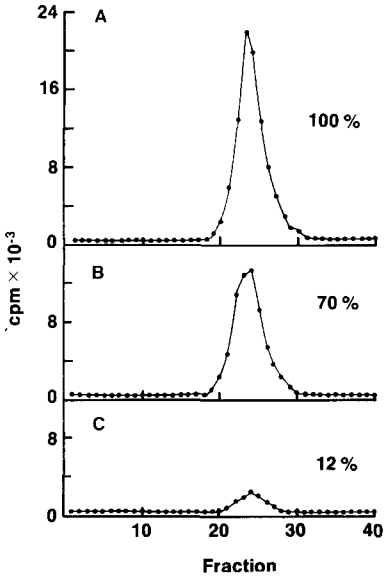

Fig. 2. Effect of aphidicolin on DNA synthesis in uninfected Vero cells. Cellular DNA was labelled with ortho[^{32}P]phosphate as described in Materials and Methods. [^{3}H]Thymidine-labelled DNA from untreated cells was used as internal density marker (not shown). A. Control. B. 1 μM aphidicolin. C. 10 μM aphidicolin.

TABLE 2

Inhibition of HSV-1 and cellular DNA synthesis by aphidicolin and foscarnet (%)

Compound	HSV-1 infected cells		Uninfected cells
	HSV-1 DNA	Cell DNA	Cell DNA
10 μM aphidicolin	100	82	94
250 μM foscarnet	85	16	21
10 μM aphidicolin +250 μM foscarnet	100	83	98

Discussion

The selectivity of an antiherpes compound can be determined directly by measuring the amount of viral and cellular DNA synthesized in the presence of the inhibitor [1–3]. By comparing the effect of a compound on cellular DNA synthesis in infected and uninfected cells one could also determine if the inhibitor is selectively activated in infected cells.

To determine if the cellular DNA synthesis in both HSV-1 infected and uninfected Vero cells is mainly DNA polymerase α dependent, we used the known DNA polymerase α-specific inhibitor, aphidicolin [4–7]. This compound inhibited HSV-1 DNA

synthesis to 50% at 0.07 μM and cellular DNA synthesis to 50% at 2.1 μM in infected Vero cells and at 1.3 μM in uninfected Vero cells (Table 1). The results indicate that the cellular DNA synthesis found in HSV-1 infected or uninfected Vero cells was mainly DNA polymerase α dependent. The cell culture result is in agreement with the observation that purified HSV-1 DNA polymerase is more sensitive to aphidicolin than DNA polymerase α and that DNA polymerase β and γ are not sensitive [4]. However, we found a higher selectivity in the inhibition of HSV-1 DNA synthesis when compared to inhibition of cellular DNA synthesis in the infected cells.

Foscarnet has been shown to be a selective inhibitor of HSV-1 DNA synthesis [2]. The cellular DNA synthesis which was only slightly affected by 250 μM foscarnet, which inhibited the HSV-1 DNA synthesis to 85%, was sensitive to aphidicolin (Table 2). This result suggests that the DNA synthesis remaining after foscarnet treatment in infected and uninfected Vero cells was mainly DNA polymerase α dependent.

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