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PRELIMINARY COMMUNICATIONS

INHIBITION OF HTLV-III/LAV REPLICATION BY FOSCARNET

Prem S. Sarin,* Yoshitaka Taguchi, Daisy Sun, Arthur Thornton, Robert C. Gallo and Bo Öberg§

Lab. of Tumor Cell Biology, National Cancer Institute, Bethesda, MD 20205, USA; and §Department of Antiviral Chemotherapy, Astra Läkemedel AB, Södertälje, Sweden

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The discovery of a human T lymphotropic retrovirus (HTLV-III/LAV) as the likely cause of the acquired immunodeficiency syndrome (AIDS) [1,2] has presented a possible target for chemotherapy. Foscarnet (trisodium phosphonoformate, PFA) is an antiviral agent that inhibits DNA polymerases of the five human herpes viruses [3] and several reverse transcriptases [4]. Due to its similarity to pyrophosphate, foscarnet interacts with the herpesvirus DNA polymerases at a site where pyrophosphate is split off during polymerization of nucleoside triphosphates. In this report, we present the results of our study on the effect of foscarnet on HTLV-III reverse transcriptase and virus replication in cell cultures.

Materials and Methods

Source of HTLV-III Reverse Transcriptase and Assay Conditions. HTLV-III reverse transcriptase used in these studies was purified by sequential chromatography on DEAE cellulose, phosphocellulose and hydroxyapatite. The purified enzyme was stored in 50 mM Tris-HCl (pH 7.5), 1 mM dithiothreitol (DTT), 0.01% Triton X-100 and 20% glycerol. Reverse transcriptase assays were carried out in a reaction mixture (50 µl) containing 50 mM Tris-HCl (pH 7.5), 5 mM DTT, 100 mM potassium chloride, 0.01% Triton X-100 or NP40, 10 µg/ml $(dT)_{15}\cdot(A)_n$ as template primer and [^{3}H]deoxythymidine triphospahate ([^{3}H]-dTTP). The reaction mixture was incubated for 1 hr at 37°, and the reaction was stopped by the addition of 50 µg of yeast tRNA and 2 ml of 10% solution of trichloroacetic acid (TCA) containing 1 mM sodium pyrophosphate. The samples were filtered on millipore filters (0.45 µm), washed first with 5% TCA solution (5 times) and then with 2 ml of 70% ethanol. The filters were dried under a heat lamp, scintillation fluid was added and the radioactivity counted in a β-scintillation

*Address all correspondence to Dr. Prem S. Sarin, Laboratory of Tumor Cell Biology, NCI, NIH, Bldg. 37, Rm. 6B17, Bethesda, MD 20205 (USA).

counter.

HTLV-III Infection of H9 Cells. H9 cells were treated with polybrene (2 μg/ml) for 30 min at 37°C, washed free of polybrene and infected with 2 x 10^8 HTLV-III virus particles per 4 X 10^5 H9 cells. The positive control sample did not receive any drug whereas the test samples received various concentrations of foscarnet. The cultures were analyzed for HTLV-III reverse transcriptase activity as described above.

Immunofluorescence Assays. Immunofluorescence assays were carried out on methanol:acetone (1:1) fixed cells using monoclonal antibodies to HTLV-III p15 and p24. The HTLV-III infected cells with or without drug treatment were fixed on toxoplasmosis slides. After fixation with methanol-acetone (1:1) for 30 min at room temperature, the slides were stored in sealed plastic bags at -20°C until ready for use. The monoclonal antibodies were added to duplicate wells, incubated at room temperature in a humid chamber for 1 hr and washed with PBS containing 0.25% Triton X-100 for 2 hr. The cells were then exposed to fluorescein (FITC) labeled goat antimouse IgG (Capell Labs.) for 1 hr and washed with PBS buffer containing 0.25% Triton X-100 overnight. The slides were mounted with 50% glycerol and cell fluorescence observed under a Zeiss fluorescence microscope.

Results and Discussion

The effect of foscarnet on purified HTLV-III reverse transcriptase (RT) was assayed as a function of drug concentration. As shown in Fig. 1A, the concentration of foscarnet causing 50% inhibition of enzyme activity was found to be less than 2 μM. Similar results were obtained when the effect of foscarnet was studied on the endogenous reverse transcriptase activity of the disrupted virus in the absence of an exogenously added template-primer such as $(dT)_{15}\cdot(A)_n$. The inhibition of reverse transcriptase by foscarnet has been shown to be noncompetitive with respect to substrate and uncompetitive with respect to template [4].

The effect of foscarnet on the replication of HTLV-III in H9 cells was determined as a function of both foscarnet concentration and time of incubation. As shown in Figure 1B, the degree of inhibition is dependent on both the time of incubation and the foscarnet concentration. A concen-

tration of 300 μM was sufficient to obtain more than 95% inhibition after six days of incubation. No difference in the inhibition of the reverse transcriptase activity was observed if the virus pellet obtained from the cell culture supernatant fraction was washed with phosphate buffered saline (PBS) before enzyme assays, suggesting that the enzyme activity was not nonspecifically inhibited by foscarnet carried in the virus pellet from the culture medium. Table 1 shows that foscarnet also caused a reduction in the expression of the HTLV-III proteins p15 and p24. Foscarnet does not inhibit protein synthesis [3] and, therefore, the observed reduction in these viral proteins represents an inhibition of virus replication.

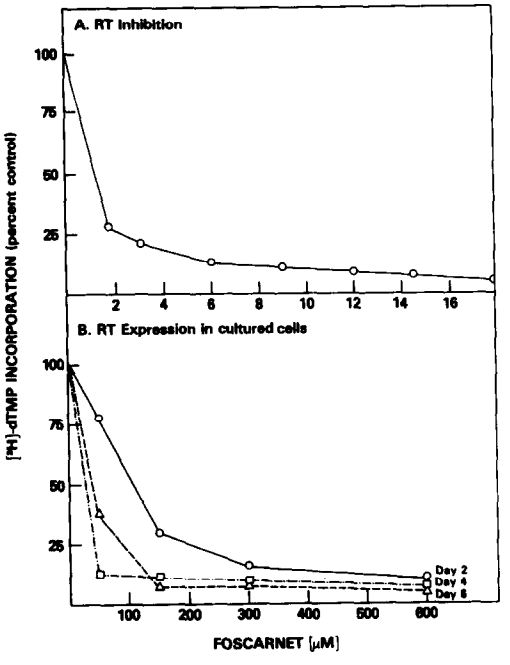

Figure 1

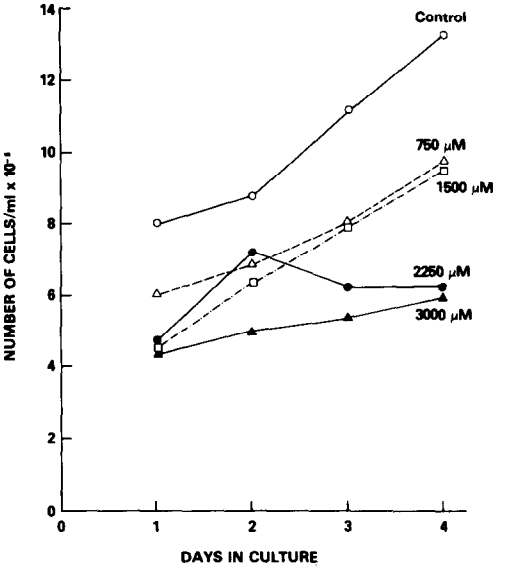

Figure 2

Fig. 1 (A) Inhibition of HTLV-III reverse transcriptase by foscarnet.

(B) Inhibition of HTLV-III replication by foscarnet.

Fig. 2 Effect of foscarnet on the growth of uninfected H9 cells.

TABLE 1. Inhibition of HTLV-III Virus expression by foscarnet as measured by monoclonal antibodies to HTLV-III p15 and p24*

Foscarnet Conc. (μM)	Percent of cells expressing HTLV-III p15 or p24					
	Day 2		Day 4		Day 6	
	p15	p24	p15	p24	p15	p24
0	10	10	46	50	48	51
30	10	10	80	60	45	45
150	4	<1	5	4	15	10
300	0	0	<1	0	5	4
600	0	0	<1	0	<1	0

*Immunofluorescence assays were carried out as described in materials and methods.

Foscarnet inhibits cell growth by 50% at concentrations of about 1000 μM in a variety of cell types [5] and this inhibition is reversible. Even when stationary cells are treated with 10 mM foscarnet, normal cell growth can be seen after removal of the drug. Uninfected H9 cells showed less than 50% inhibition at 750 μM foscarnet and more pronounced inhibition at higher concentrations as shown in Figure 2.

Foscarnet has mainly been evaluated clinically as a topical formulation against labial and genital herpes and found to be active and well tolerated [6]. It has also been given by infusion to more than 140 patients with severe herpesvirus infections mainly caused by cytomegalovirus (CMV) [7]. These patients were given foscarnet intravenously by constant infusion for 1-4 weeks and, at steady state, serum levels of foscarnet reached in most cases were 300-450 μM (100-150 μg/ml). There were clear indications of a beneficial effect in patients treated with this drug.

Due to its non-competitive and direct mode of action, it appears that the sensitivity of HTLV-III to foscarnet in cell culture may be predictive of its effect against virus replication *in vivo* [3,8]. It is thus possible to give foscarnet to patients at serum concentrations that will block HTLV-III replication and subsequent infection of T helper cell population. It is important, therefore, to evaluate the possible therapeutic effect of foscarnet against HTLV-III in patients who have not progressed to fully developed AIDS and who still have immune systems capable of cooperating

with an antiviral drug. Suramin [9] and ribavirin [10] have also been reported to inhibit HTLV-III but only at concentration half [9] or above [10] those causing cellular toxicity. Thus it is extremely important that potential efficacy of foscarnet, either alone or in combination with other drugs, against HTLV-III infection should be evaluated clinically as soon as possible in AIDS and pre-AIDS patients for possible chemotherapeutic control of the disease.

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