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INHIBITORY ACTIVITY OF THE NEW ADAMANTANE DERIVATIVE *N,N'*-BIS(ETHYLENE)-*P* (1-ADAMANTYL)-PHOSPHONIC DIAMIDE AGAINST ROUS SARCOMA VIRUS

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A new adamantane derivative *N,N'*-bis (ethylene) *P* (1-adamantyl)-phosphonis diamide, was found to inhibit Rous sarcoma virus replication in much the same manner as reported for the parent compound, 1-adamantanamine hydrochloride.

N,N'-bis(ethylene)-*P* (1-adamantyl)-phosphonic diamide Rous sarcoma virus

INTRODUCTION

N,N'-bis(ethylene)-*P* (1-adamantyl)-phosphonic diamide (NYPD) is chemically related to 1-adamantanamine hydrochloride (AdH) (Fig. 1). It has already been shown that NYDP has antileukemia and antifertility activity in mice [1]. AdH has been shown to inhibit infection by oncogenic RNA viruses [4,5,7]; it acts at an early stage of infection since a decrease in virus production was only observed when it was added prior to infection [7]. The experiments reported here were initiated in order to test the effects of NYDP on viral replication and transformation in cells infected with Rous sarcoma virus (RSV). The cells used were chick embryo fibroblasts (CEF) and the RSV-transformed non-producer rat kidney cell lines, LA-31-NRK and B77-NRK.

EXPERIMENTAL

The procedures for cell culture were those described previously [2,3]. Wild strains of RSV were Prague-A (PR-A), Schmidt-Ruppin-D (SR-D), and Bratislava-77 (B77), all obtained from Dr. P.K. Vogt (Dept. of Microbiology, University of Southern California School of Medicine, Los Angeles). NYPD is one of a series of compounds prepared in the laboratory of one of us (L.A. Cates).

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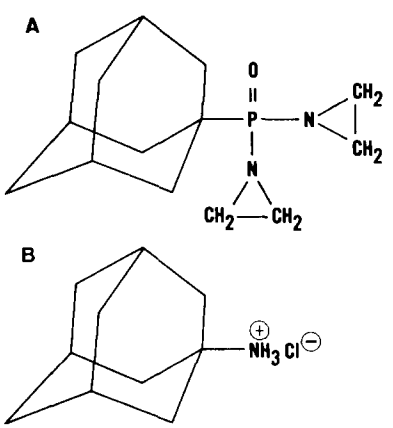
Cells were counted on a Hycell-300 cell counter (Hycel Co., Houston, Texas). Virus was applied at about 100,000 focus-forming units (f.f.u.) per culture. Virus titers were determined by focus assay [2,3]. Morphological transformation was evaluated by visual observation using an inverted microscope. Dead cells were removed using 0.05% trypsin before viable cells were collected with 0.2% trypsin.

Prior to evaluation of the anti-RSV activity of NYPD, its effect on cell growth was tested (Fig. 2). At low concentrations of 25 μg/ml, it did not significantly inhibit the multiplication of CEF; at 50 μg/ml, however, significant inhibition was observed. This level of cytotoxicity is the same as that reported for AdH [4,5]. A direct virucidal effect of NYPD on RSV was ruled out by experiments in which a virus suspension was incubated in the presence of NYPD and was then tested for live virus by focus assay. In this experiment, NYPD had no direct inactivating effect on RSV. In this respect, NYPD is also similar to AdH [4,5]. Inhibition of adsorption of RSV to CEF was considered a possible, early site of action of NYPD. In order to test this hypothesis, monolayer cultures were exposed to virus at 39°C in the presence or absence of 25 μg/ml NYPD. At different time intervals, the unadsorbed virus was assayed. These experiments did not show any effect of NYPD on adsorption of RSV to CEF. Again these results paralleled the findings of others [4,6] using AdH.

Previous reports [4,7] have suggested that the AdH-susceptible process takes place in the early stages of viral replication. Therefore, experiments were initiated to see whether this was also the case for NYPD. Table 1 shows the results of an experiment in



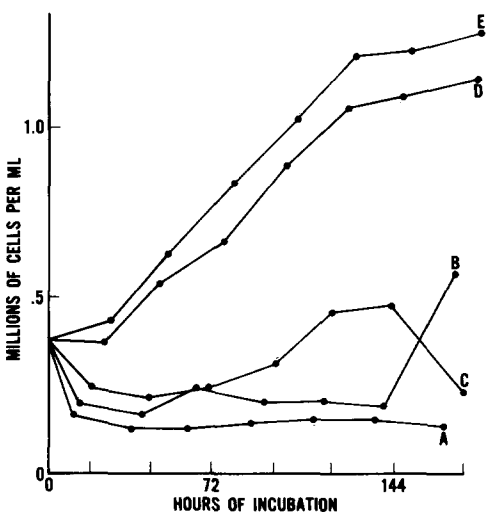


Fig. 1. Chemical structure of *N,N'*-bis(ethylene)-*P* (1-adamantyl)-phosphonic diamide (A) and 1-adamantanamine hydrochloride (B).

Fig. 2. Growth curves of uninfected CEF in the presence of NYPD. Concentrations (μg/ml) applied: A) 500; B) 100; C) 50; D) 25; E) control.

TABLE 1

Inhibition by NYPD of RSV infection in CEF: effect of time of application of NYPD

Time of addition of NYPD (25 μg/ml) relative to time of inoculation of RSV (min)	Virus yield (f.f.u./μl) obtained 7 days after infection with RSV-strain		
	B77	PR-A	SR-A
Control (no NYPD)	64	69	36
−30	0.75	0.27	0.46
−10	0.69	0.32	1.8
0	13	15	18
+10	66	71	58
+30	69	75	72

which NYPD was added to CEF cultures at different time points relative to infection with RSV. As in the study of the action of AdH on fowl plague virus [5], NYPD remained present in the culture medium throughout the incubation period. It can be seen that NYPD was only effective when applied before infection. The requirement for NYPD to be introduced before virus infection may be due to its acting on an early event in virus replication or to slow diffusion of NYPD into the intracellular compartments. However, the fact that NYPD, applied at higher concentrations, still did not act after viral infection had taken place pleads against this possibility.

Experiments were also done to test the possibility that NYPD would affect the expression of integrated RSV genomes. This was done by assessing the degree of transformation in two RSV-transformed non-virus-producing [3] rat cell lines, LA-31-NRK and B77-NRK, during incubation with NYPD. LA-31-NRK was originally transformed with a temperature-sensitive mutant of RSV [3]; and B77-NRK was originally transformed by the wild-type RSV-strain B77. When cultured at the permissive temperature (33°C), LA-31-NRK cells exhibit the morphological characteristics of the transformed state, whereas at non-permissive temperature (39°C), they exhibit normal characteristics. B77-NRK cells show morphological changes associated with transformation at all temperatures of culture. In experiments where B77-NRK cells were incubated at 33°C or 39°C, the transformed state was expressed in the absence as well as in the presence of NYPD (concentrations ranging from 10 to 50 μg/ml). Similarly, the LA-31-NRK cell line expressed its transformed state at the permissive temperature (33°C), irrespective of whether NYPD was present in the culture medium. Moreover, the transition from the untransformed to the transformed state, induced by a 39–33°C temperature shift, was not inhibited by the presence of NYPD.

In conclusion, our experiments with RSV-infected CEF indicate that NYPD affects RSV in much the same manner as reported for the chemically related substance AdH, i.e. by inhibiting a relatively early event in virus replication, subsequent to adsorption of

the virus to the cell membrane. The data obtained from experiments on RSV-transformed non-producer cells indicate that NYPD is unable to affect the expression of the oncogenes carried by the incorporated viral genomes.

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