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Selective inhibition of arthropod-borne and arenaviruses in vitro by 3′-fluoro-3′-deoxyadenosine

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Summary

A novel nucleoside analog, 3′-fluoro-3′-deoxyadenosine (3′F3′dAdo), was evaluated for antiviral activity against several arthropod-borne and arenaviruses in Vero cell culture. The following 50% inhibitory concentrations (EC_{50}) of virus plaque formation were obtained against the test viruses: Semliki Forest (10.3 μM) and Venezuelan equine encephalitis (5.3 μM) alphaviruses, lymphocytic choriomeningitis (7.7 μM) and Pichinde (>32 μM) arenaviruses, Punta Toro (>32 μM) and San Angelo (1.6 μM) bunyaviruses, banzi flavivirus (4.0 μM), and Colorado tick fever orbivirus (0.6 μM). By comparison, the broad-spectrum antiviral agent ribavirin was active against lymphocytic choriomeningitis (18 μM), Pichinde (24 μM), Punta Toro (114 μM), and San Angelo (99 μM) viruses, but was less active against the other 4 viruses (>200 μM). Vero cell proliferation and thymidine and uridine incorporation into replicating Vero cells were inhibited by 50% with 3′F3′dAdo concentrations of 36, 45, and 32 μM, respectively. In virus yield reduction assays, increasing the multiplicity of infections of Semliki Forest and Venezuelan equine encephalitis viruses reduced the inhibitory activity of 3′F3′dAdo. Using the same assay, 3′F3′dAdo was found to enhance Punta Toro virus replication up to 5-fold relative to the untreated control. By adding the nucleoside transport inhibitor nitrobenzylthioinosine (100 μM) to the culture medium, antiviral activity against the two alphaviruses was eliminated, indicating that 3′F3′dAdo uses the nucleoside transport system for cell entry. When actinomycin D (5 μM) was used to greatly suppress cellular RNA synthesis in Semliki Forest virus-infected

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and uninfected cells, 3′F3′dAdo preferentially inhibited viral RNA synthesis. The results of these studies indicate 3′F3′dAdo is a selective inhibitor of most of the viruses tested and should be a promising candidate for in vivo evaluations.

Antiviral; Adenosine analog; Togavirus; Bunyavirus; Flavivirus; Nucleoside transport

Introduction

3′-Fluoro-3′-deoxyadenosine (3′F3′dAdo) is a newly reported broad-spectrum inhibitor of several different DNA and RNA viruses in cell culture (Van Aerschot et al., 1989; Mikhailopulo et al., 1991), and was also found active in a vaccinia virus tail lesion model in mice (Van Aerschot et al., 1989). The spectrum of viruses inhibited is unique and differs from that exhibited by adenosine analogs whose cellular target is S-adenosylhomocysteine hydrolase. Because the primary reports indicated 3′F3′dAdo inhibited Semliki Forest virus, an arthropod-borne alphavirus, we expanded the studies of the previous investigations to include other types of arthropod-borne (alpha-, bunya-. flavi-, and orbiviruses) and arenaviruses. The diseases they cause in humans can be quite severe and sometimes fatal.

To date, few potent inhibitors of these viruses have been identified, and most of these agents inhibit cell replication at doses at or near antiviral concentrations. Some of these substances include ribavirin (Sidwell et al., 1988), ribavirin 5′-sulfamate (Smee et al., 1988), a newly reported EICAR analog related to ribavirin (De Clercq et al., 1991), various anti-arenavirus agents (Burns et al., 1988; Andrei and De Clercq, 1990), and other nucleosides (Goebel et al., 1982; Huggins et al., 1984; Smee et al., 1987). The results of the present study show 3′F3′dAdo to be a potent inhibitor of certain alphaviruses, arenaviruses, bunyaviruses, flaviviruses, and orbiviruses at concentrations which are not cytotoxic.

Materials and Methods

Compounds

3′F3′dAdo was synthesized at Rega Institute by the published procedure (Van Aerschot et al., 1989). Ribavirin, a broad-spectrum antiviral agent used as a positive control (Sidwell et al., 1972), was obtained from Viratek, Costa Mesa, CA. Nitrobenzylthioinosine was purchased from Sigma Chemical Co., St. Louis, MO. Actinomycin D and MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] were from ICN Biochemicals, Cleveland, OH. [^{3}H]Adenosine and [^{3}H]2′-deoxyadenosine were obtained from ICN Radiochemicals, Irvine, CA.

Viruses and cells

Semliki Forest virus (original strain), Venezuelan equine encephalitis virus (Trinidad, TC attenuated strain), lymphocytic choriomeningitis virus (Armstrong E-350 strain), San Angelo virus, banzi virus (H336 strain), Colorado tick fever virus (Florio strain), and Vero 76 cells were obtained from the American Type Culture Collection, Rockville, MD. Pichinde virus (An 4763 strain) was from David Gangemi, University of South Carolina School of Medicine, Columbia, SC. Punta Toro virus was acquired from the U.S. Army Medical Research Institute of Infectious Diseases, Ft Detrick, MD. The cells were grown in Eagle's medium supplemented with 10% fetal bovine serum (FBS), 0.1% $NaHCO_3$, and 50 μg gentamicin/ml. Routine mycoplasma testing gave negative results in gentamicin-free cultures.

Plaque and virus yield reduction assays

Vero cells have been used by other researchers to plaque most of the viruses (Buchmeier et al., 1980; Huggins et al., 1984; Burns et al., 1988), and we found by experimentation that all of the viruses in this study could be plaqued. The plaque assay method of Smee et al. (1983) was used. Virus was adsorbed on 6-well plates of Vero cells overlayed with medium containing 2% FBS and 0.5% SeaPlaque agarose (FMC Corp., Rockland, ME), and were incubated for varying time periods to allow plaque formation. 3'F3'dAdo or ribavirin was applied to cells as part of the agar-containing medium after the 1-h virus adsorption period. Semliki Forest virus was incubated for 2 days, Pichinde virus for 5 days, and all other viruses for 4 days. After fixing the plates for 15 min with 10% buffered formalin, the agar was removed and the cells stained with 0.2% crystal violet in 20% ethanol. The plaques were counted with the aid of a Plaque Viewer (Bellco Glass Co., Vineland, NJ). Fifty percent inhibitory concentrations (EC_{50}) of 3'F3'dAdo were estimated by plotting concentration versus percent inhibition on semi-log paper.

Virus yield reduction assays using different multiplicities of virus infection (MOI) were performed as described previously (Smee et al., 1988). This entailed adding the virus and 3'F3'dAdo (at varying concentrations) simultaneously to Vero cells in 24-well plates. The virus was harvested and frozen when the untreated culture wells were completely destroyed by Semliki Forest or Venezuelan equine encephalitis virus. In the case of Punta Toro virus, 3'F3'dAdo enhanced virus replication compared to the untreated control, so virus replication was terminated when wells containing 2 μM 3'F3'dAdo were destroyed completely by virus. Virus titers in the samples were then determined by plaque assay as described above.

Cytotoxicity assays

To determine the effects of 3'F3'dAdo on Vero cell proliferation, approximately 1×10^4 cells were placed in each well of 24-well plates in growth medium containing various concentrations of compound. After 3 days, the determination of cell numbers was made indirectly using the MTT assay

as described (Raju et al., 1989). This necessitated transferring 100 μl volumes from each well to a 96-well plate to determine optical density with an ELISA plate reader. The use of 24-well plates for this assay is preferred over 96-well plates, because in our experience the well-to-well variability in 24-well plates is less. [^{3}H]Thymidine, [^{3}H]uridine, and [^{3}H]leucine (1 μCi/ml each) were used to monitor DNA, RNA, and protein syntheses, respectively, in uninfected cells co-treated with 3′F3′dAdo. These toxicity determinations were made in replicating cells (8×10^4 cells/well in 24-well plates). 3′F3′dAdo was added to the cell medium 4 h before a 1-h radioactivity pulse to allow time for the antiviral nucleoside to become phosphorylated to the active form. The basic methods for counting acid-soluble and insoluble fractions of radiolabeled cells have been described previously (Smee et al., 1983) with slight modifications. The acid-insoluble counts were first precipitated on the bottom of the 24-well plates using 0.4 N perchloric acid. This treatment released the acid-soluble radioactivity which could be counted directly. The acid-insoluble counts, representing DNA, RNA and protein of the cell, were solubilized off the bottom of the plates using 0.5 N KOH. Because we found that the hydroxide solution may give non-specific counts by liquid scintillation spectroscopy, vials containing these samples were neutralized with an excess of 0.5 N acetic acid prior to counting. Although KOH will degrade the RNA, the counts measured when [^{3}H]uridine was used originally were in the precipitated RNA.

The radioactivity methods described here were also used to assess whether 3′F3′dAdo could block the uptake of [^{3}H]nucleosides into cells. Confluent monolayers of cells were treated simultaneously with 3′F3′dAdo and [^{3}H]nucleoside for 1 h. Then the medium was removed, the cells were quickly washed once with medium, and the aspirated wells were treated with 0.4 N perchloric acid. The radioactivity in the perchloric acid fraction was then counted.

Semliki Forest virus RNA synthesis assay

Actinomycin D has been reported to suppress cellular RNA synthesis in Semliki Forest virus infected cells, allowing detection of only viral RNA synthesis (Martin and Burke, 1974). To be able to use this technique, it was first necessary to determine that actinomycin D did not eliminate the antiviral activity of 3′F3′dAdo, since it is known to eliminate the activity of the antiviral agent ribavirin (Malinoski and Stollar, 1980). By plaque assay methods, actinomycin D (5 μM) had no effect on plaque numbers or sizes in wells treated with concentrations of 3′F3′dAdo ranging from 1 to 64 μM. To assess the effect of 3′F3′dAdo on RNA synthesis in actinomycin D-treated cultures, confluent Vero cell monolayers in 24-well plates were left uninfected or were infected with Semliki Forest virus (3 MOI [see Fig. 1 for explanation] for 1 h). Cultures were pre-treated 2 h with 3′F3′dAdo to allow time for uptake of the compound into cells and possible intracellular metabolism to an active form. This was deemed important since the cells were challenged with a high virus MOI, and the antiviral effect of 3′F3′dAdo will be shown in this report to be decreased as the

MOI increases. Wells were then pulse labeled with [^{3}H]uridine (2 μCi/ml) from 4 to 6 h after virus adsorption, with 3'F3'dAdo present during the entire time of the experiment. Quantitation of radioactivity from the acid-insoluble fraction of cells was done as described above.

Results

Antiviral and anti-cellular activities

3'F3'dAdo was inhibitory to most of the arthropod-borne and arenaviruses listed in Table 1, with the exception of Punta Toro and Pichinde viruses where no antiviral effect was observed at 32 μM (which was the highest concentration tested; concentrations of 64 and 128 μM began to be overtly toxic to cells under agar). Colorado tick fever virus was the most sensitive virus to inhibition by the compound. Vero cell proliferation was inhibited at 36 μM, which was higher than the concentrations that inhibited most of the viruses. Where antiviral activity was evident, selectivity indices ranged from 3.5 to 60. By comparison, the broad-spectrum antiviral agent ribavirin was less active against these viruses (Table 1). Ribavirin showed approximately the same degree of activity against lymphocytic choriomeningitis and Pichinde arenaviruses (18 and 24 μM, respectively), and against Punta Toro and San Angelo bunyaviruses (114 and 99 μM, respectively).

Biochemical cytotoxicities performed with ^{3}H-labeled substrates were performed in actively growing Vero cells. 3'F3'dAdo inhibited the incorporation of thymidine, uridine, and leucine by 50% at 45, 32, and >64 μM,

TABLE 1

Antiviral activities of 3'F3'dAdo and ribavirin in Vero cells

Virus or cell	Virus family	EC_{50}[a] or IC_{50}[b] (μM)		3'F3'dAdo selectivity index[c]
		Ribavirin	3'F3'dAdo	
Semliki forest	Alphavirus	>250	10.3 ± 1.2[d]	3.5
Venezuelan equine encephalitis	Alphavirus	>250	5.3 ± 1.2	6.8
Lymphocytic choriomeningitis	Arenavirus	18 ± 4.5	7.7 ± 3.1	4.6
Pichinde	Arenavirus	24 ± 6.0	>32	<1
Punta Toro	Bunyavirus	114 ± 60	>32[e]	<1
San Angelo	Bunyavirus	99 ± 32	1.6 ± 0.5	22.4
Banzi	Flavivirus	>250	4.0 ± 1.8	9.0
Colorado tick fever	Orbivirus	208 ± 107	0.6 ± 0.2	60.0
Vero cells	–	–[f]	36 ± 4.3	–

[a] Concentration inhibiting virus plaque numbers by 50%.
[b] Concentration inhibiting replicating cell numbers by 50%.
[c] Cell IC_{50} divided by virus EC_{50}.
[d] Standard deviation for 3 independent assays.
[e] Treatment with 3'F3'dAdo enhanced virus replication. See text.
[f] Not determined.

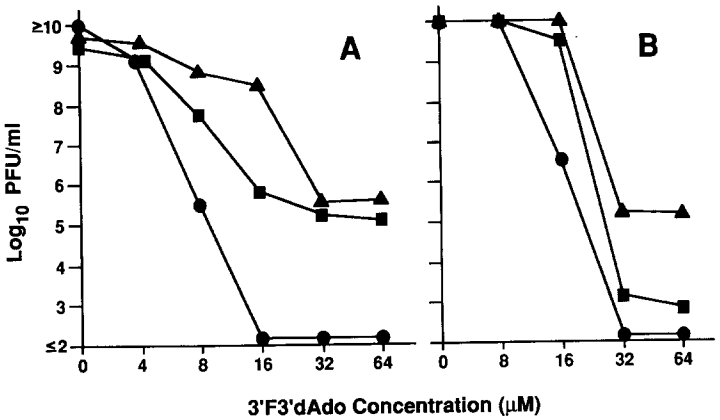

Fig. 1. Effect of multiplicity of virus infection (MOI) on the antiviral activity of 3'F3'dAdo against Venezuelan equine encephalitis (A) and Semliki Forest (B) virus infections in Vero cells. Virus titers are expressed as $\log_{10}$ plaque forming units (PFU) per ml. Symbols: ▲, MOI = 0.1; ■, MOI = 0.001; ●, MOI = 0.00001.

respectively. 3'F3'dAdo ($\leqslant 64$ μM) had no effect on the uptake of these ^{3}H-labeled materials into acid-soluble fractions of cells. The 50% inhibitory concentrations of the compound on thymidine and uridine incorporation compared favorably with that observed in the cell proliferation assay (Table 1). It was also observed that much lower concentrations of 3'F3'dAdo exhibited substantial inhibitory effects on [^{3}H]uridine incorporation in uninfected cells. Degrees of inhibition of 11, 18, 32, and 39% were evident at 3'F3'dAdo concentrations of 1, 2, 4, and 8 μM, respectively. These concentrations were within the range of those affecting the replication of many of the viruses.

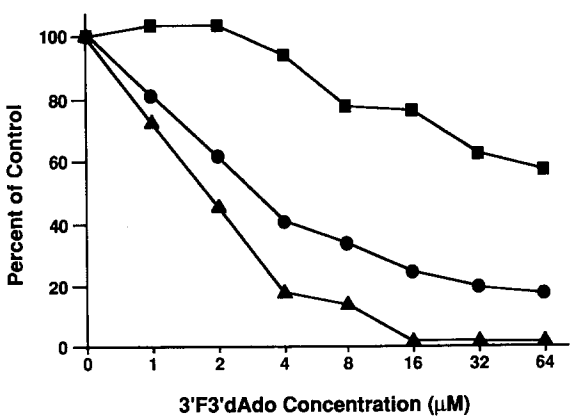

Fig. 2. Inhibition of Semliki Forest virus and cellular RNA syntheses by 3'F3'dAdo in actinomycin D (5 μM) treated cells. Counts of radioactivity in 3'F3'dAdo-treated cultures were converted to percentages of the respective untreated control (i.e., cultures devoid of 3'F3'dAdo). Symbols: ■, uninfected cells; ●, virus-infected cells; ▲, plot obtained by subtracting counts per min (cpm) in uninfected cultures from cpm in infected cells at each 3'F3'dAdo concentration prior to converting to a percentage of the control.

The effects of 3′F3′dAdo on virus production and the influence of virus MOI on antiviral activity were investigated (Fig. 1). At least a 90% (1 $\log_{10}$) reduction in virus titer occurred near the plaque reduction EC_{50} value reported in Table 1 against Semliki Forest and Venezuelan equine encephalitis viruses at the lowest MOI (10^{-5} infecting viruses per cell). The degree of virus yield reduction was input virus titer-dependent, since a greater antiviral effect was observed at 10^{-5} MOI than occurred at 10^{-1} or 10^{-3} MOI. These results were also important to establish that anti-Semliki Forest virus activity (at 32 and 64 μM 3′F3′dAdo) was present using 10^{-1} MOI prior to conducting the mode of action studies under even higher MOI conditions (see Fig. 2).

Against Punta Toro virus, initially it was observed that plaque sizes were up to 10 times larger in area in 3′F3′dAdo-containing wells than in untreated wells. The peak effect was evident at a concentration of 2 μM, where virus yield titers were 5-fold greater than the respective untreated control. Concentrations of 3′F3′dAdo below (0.5 and 1 μM) and above (4 and 8 μM) the 2 μM concentration showed 2- to 3-fold increases in virus titer. A similar enhancement of Punta Toro virus replication was seen in MA-104 cells (another type of African green monkey kidney cell line), with a 4-fold enhancement of Punta Toro virus replication occurring at 8 μM, and 2-fold enhancement at 2 and 4 μM. No enhancement nor inhibition of Punta Toro virus replication occurred in the C127I mouse cell line treated with 3′F3′dAdo, however, suggesting that the phenomenon is cell line-dependent.

Nucleoside transport studies

Two types of experiments were conducted to determine the mode of transport of 3′F3′dAdo into cells. The first involved studying the effect of the nucleoside analog to block the transport of [^{3}H]adenosine and [^{3}H]2′-deoxyadenosine into the acid-soluble fraction of cells in a 2 h labeling period. The rationale was that if 3′F3′dAdo blocked cellular entry of these related compounds, this would suggest a common pathway for cell uptake. Using 3′F3′dAdo concentrations as high as 128 μM to compete against 0.1 μM adenosine or 2′-deoxyadenosine, 12% inhibition of adenosine and 17% inhibition of 2′-deoxyadenosine uptake were observed. These results indicate that 3′F3′dAdo is either a very poor competitor with the natural nucleosides for nucleoside transport, or else the analog primarily uses an alternative pathway for cell entry. As positive controls for these studies, non-radioactive adenosine blocked the uptake of 0.1 μM [^{3}H]adenosine by 50% at 12 μM, whereas non-radioactive 2′-deoxyadenosine showed only a 30% inhibition of [^{3}H]adenosine uptake at 128 μM. Conversely, both non-radioactive adenosine and 2′-deoxyadenosine were equipotent to inhibit [^{3}H]2′-deoxyadenosine uptake into cells, with both showing a 50% inhibitory concentration of 16 μM. In follow-up studies with 3′F3′dAdo, concentrations as high as 128 μM did not block the entry of ^{3}H-labeled guanosine, 2′-deoxyguanosine, cytidine, 2′-deoxycytidine, uridine, or 2′-deoxyuridine (each at 0.1 μM) into cells.

A more direct method of determining a mode of cell entry for 3′F3′dAdo was

to use the nucleoside transport blocking agent nitrobenzylthioinosine (Plagemann et al., 1988) in a competitive manner. The concentration of nitrobenzylthioinosine (100 μM) was predetermined to inhibit the uptake of 5 μM [^{3}H]adenosine into cells by 80% in a 2-h assay. With nitrobenzylthioinosine in the culture medium, the same relative numbers of Semliki Forest and Venezuelan equine encephalitis virus plaques were present in wells containing $\leqslant$40 μM 3′F3′dAdo as were present in untreated cultures. Wells containing only 3′F3′dAdo showed complete suppression of virus plaque formation at $\geqslant$8 μM concentration. These results indicate 3′F3′dAdo uses the nucleoside transport system for cell entry.

Selective inhibition of Semliki Forest virus RNA synthesis

In actinomycin D-treated cultures, 3′F3′dAdo had a greater inhibitory effect on Semliki Forest viral RNA synthesis (under a high MOI [3 PFU/cell] condition) than on RNA synthesis in uninfected cells (Fig. 2). Also presented on the figure is a plot of the inhibition of incorporated radioactivity derived by subtracting uninfected cell counts from virus-infected cell counts (triangles). It is assumed that due to the inhibition of viral RNA synthesis, some cellular RNA synthesis would occur, particularly at the higher concentrations of 3′F3′dAdo. Thus, by subtracting out counts probably attributable to cellular RNA synthesis, the lower plot of the figure may more accurately reflect the extent of inhibition of viral RNA synthesis by 3′F3′dAdo.

Discussion

3′F3′dAdo was shown to be a potent inhibitor of all viruses tested except for Pichinde and Punta Toro viruses. It is noteworthy that of the two arenaviruses and the two bunyaviruses tested, one virus in each group was inhibited while the other was not. In contrast, ribavirin was found to inhibit the arenaviruses or the bunyaviruses to the same degree. Lymphocytic choriomeningitis arenavirus is classified differently from Pichinde virus, the former being an 'old world' virus related to Lassa fever virus, and the latter a 'new world' virus in the Tacaribe virus complex (Bishop, 1990a). The two viruses are also distinguishable by antibody neutralization tests (Andrewes et al., 1978). Punta Toro virus is in the *Phlebovirus* group of bunyaviruses (Sidwell et al., 1988), whereas San Angelo virus is in the California group (Sather and Hammon, 1967). Bunyaviruses are a heterogeneous collection of different virus subgroups classified on the basis of having a tripartate genome and a lipid envelope (Bishop, 1990b). Thus, it may not be surprising to find differences in susceptibility to a particular antiviral agent among viruses of the same family. Even very closely related viruses may react differently to inhibitors. For example, herpes simplex virus type 1 is very sensitive to inhibition by bromovinyldeoxyuridine, whereas herpes simplex virus type 2 is resistant (De Clercq et al., 1979). Influenza B virus is inhibited by 6-azauridine whereas

influenza A virus is resistant (Smee et al., 1987). These results emphasize the need to evaluate novel antiviral substances against each type of virus of interest, instead of making projections of activity based upon selected 'indicator' viruses.

Cellular toxicity induced by 3'F3'dAdo occurred at concentrations higher than those affecting replication of all viruses except Pichinde and Punta Toro viruses. There was not a great degree of selectivity toward virus inhibition, however, if one considers the 30% inhibitory effect of 3'F3'dAdo on [^{3}H]uridine incorporation into cellular RNA occurring at a 4 μM concentration. However, the results clearly demonstrated that viral RNA synthesis was preferentially inhibited over cellular RNA synthesis in Semliki Forest virus-infected cells. 3'F3'dAdo was also more inhibitory to cellular RNA synthesis in replicating cells than in resting cell monolayers (comparing percentage inhibition values reported in the text to data in Fig. 2).

The potent efficacy of 3'F3'dAdo against many of these viruses appears to be greater than that achieved by all other compounds reported to date. 3'F3'dAdo is more active than ribavirin and 3-deazaguanine against Colorado tick fever virus (Smee et al., 1981a), and is superior to ribavirin and/or tiazofurin against banzi, San Angelo, and Venezuelan equine encephalitis viruses (Huggins et al, 1984; Smee et al., 1987). Selenazofurin (Huggins et al., 1984) and 3'F3'dAdo appear to be equally potent against Venezuelan equine encephalitis virus, although selenazofurin is more cell-inhibitory. Ribavirin 5'-sulfamate (Smee et al., 1988) and the novel EICAR analog (De Clercq et al., 1991) show potencies comparable to that of 3'F3'dAdo against Semliki Forest virus, the former two compounds being more cytotoxic, however. Ribavirin and selenazofurin are both active against Pichinde virus (Huggins et al., 1984; Burns et al., 1988), whereas 3'F3'dAdo is inactive. Andrei and De Clercq (1989) have also tested numerous compounds against arenaviruses although the virus types were different than those evaluated here, so direct comparisons of relative potencies of compounds cannot be made.

The decrease in antiviral activity with increasing MOI reported here is similar to the effect reported in other investigations (Smee et al., 1981b). At a lower MOI the virus has to undergo more cycles of replication, compounding the inhibitory drug effect, whereas a higher MOI causes a greater virus burden (i.e., the infectious viral RNA) that has to be contended with by the antiviral agent. Even though we anticipated that 3'F3'dAdo would be less effective under high MOI conditions, this may not always be the case with virus inhibitors. For example, in similar studies comparing ribavirin to amantadine against high and low influenza virus MOI, ribavirin retained much of its potency at high MOI, whereas amantadine did not (Browne et al., 1983). There may be clinical implications associated with the performance of a compound under different MOI conditions. Early in the course of infection the overall virus burden would be less, and the disease may be easier to treat, whereas later in the disease when larger concentrations of virus may infect new cells (i.e, higher MOI conditions) the antiviral agent may prove less effective (Browne et al., 1983).

The enhancement of virus titer in Punta Toro virus-infected cells treated with 3′F3′dAdo is interesting, but not unique to metabolic inhibitors. For example, ribavirin was shown to enhance the replication of lymphocytic choriomeningitis virus at certain concentrations (Gessner and Lother, 1989), and actinomycin D is able to stimulate the replication of respiratory syncytial virus (Lambert et al., 1980). There probably is a general mode of action by which certain compounds enhance virus replication. Since viruses and cells compete for the same intracellular substrates and ribosomal machinery, any inhibitor that will preferentially affect cell replication will give the virus a greater advantage to replicate itself. The specific mode of stimulation of Punta Toro virus replication by 3′F3′dAdo is not known, and will require further investigation.

These studies provide evidence that 3′F3′dAdo uses the nucleoside transport system for cell entry, based upon the observation that antiviral activity was eliminated in the presence of nitrobenzylthioinosine, a nucleoside transport inhibitor (Plagemann et al., 1988). Because of the poor ability of 3′F3′dAdo to block the uptake of adenosine and 2′-deoxyadenosine into cells, we initially hypothesized that 3′F3′dAdo entered cells by an alternative pathway. Such was shown to be the case for another 3′-deoxynucleoside, 3′- azido-3′-deoxythymidine, which enters cells by non-facilitated diffusion (Zimmerman et al., 1987). In order to study the transport of 3′F3′dAdo in more detail, it will be necessary to obtain the compound in a radioactive form.

We initially thought that using non-radioactive nucleosides such as 3′F3′dAdo, adenosine and 2′-deoxyadenosine to block the uptake of [^{3}H]adenosine and [^{3}H]2′-deoxyadenosine would be a fairly straight forward approach to determine a common pathway for entry into cells. This turned out not to be the case as was demonstrated by the way each competing substance behaved. Initially it seemed puzzling that it took over 10 μM non-radioactive adenosine or 2′-deoxyadenosine to cause a 50% reduction in uptake of the respective 0.1 μM [^{3}H]nucleoside. If simple competition were involved, then 0.1 μM of the non-radioactive nucleoside would have inhibited uptake by 50%. However, the nucleoside transport system takes more compound into the cells as the extracellular concentration of adenosine or 2′-deoxyadenosine increases. It is only at saturation of this capacity that any further extracellular concentration will 'dilute out' the [^{3}H]nucleoside entering the cells. This accounts for the high concentrations of non-radioactive natural nucleosides required to block uptake of the ^{3}H-labeled nucleosides. This also raises the possibility that 3′F3′dAdo caused a stimulation of [^{3}H]adenosine and [^{3}H]2′-deoxyadenosine uptake into cells, thus masking a possibly more pronounced competitive effect. Another factor to consider in these studies is the intracellular conversion of the nucleosides inside the cells (such as adenosine to 2′-deoxyadenosine) and the effect this may have to block uptake of the [^{3}H]nucleoside. Thus, non-radioactive adenosine may have inhibited [^{3}H]2′-deoxyadenosine uptake by competing with extracellular transport as well as by inhibiting through an intracellular feedback mechanism. These studies serve to point out the difficulties that may be encountered in interpreting results from what otherwise appear to be simple competitive assays.

Since 3'F3'dAdo is an analog of adenosine, it is likely that the compound undergoes phosphorylation, and one of the phosphorylated derivatives inhibits viral (and cellular) RNA synthesis. Van Aerschot et al. (1989) have ruled out the unphosphorylated nucleoside itself as being an inhibitor of S-adenosylhomocysteine hydrolase, a cellular enzyme which when inhibited leads to inhibition of certain viruses. We know that 3'F3'dAdo does not inhibit Semliki Forest virus and Venezuelan equine encephalitis virus adsorption and penetration, since incubation of virus with the agent prior to and during cell exposure (followed by removal of the compound after the virus adsorption/penetration period) did not lead to a decrease in virus plaque numbers relative to untreated controls (unpublished results).

The infections caused by arthropod-borne and arenaviruses have a rapid onset and high fatality rate in rodents (Sidwell et al., 1988, Smee et al., 1990). The animals generally recover and remain healthy if they survive the acute infection for two weeks. For this reason, long-term antiviral treatments (using compounds that do show in vivo efficacy) are not required to provide protection to the animals. Thus, even though some cellular toxicity may be evident, a short course of therapy may be beneficial to the host. 3'F3'dAdo has already been shown to have activity in a tail lesion model of vaccinia virus infection (Van Aerschot et al., 1989). Encephalitis (which many of these viruses cause) may be more difficult to treat due to the generally low ability of nucleosides to penetrate the blood–brain barrier. Since 3'F3'dAdo arrests viral disease in some of these arthropod-borne and arenavirus infections, further in vivo studies appear to be warranted.

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