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ANTI-VIRAL ACTIVITY OF 3-DEAZAADENOSINE AND 5'-DEOXY-5'-ISOBUTYLTHIO-3-DEAZAADENOSINE (3-deaza-SIBA)

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SUMMARY

3-Deazaadenosine and 5'-deoxy-5'-isobutylthio-3-deazaadenosine (3-deaza-SIBA) inhibits replication of both herpes simplex type 1 virus and the RNA type C virus, HL-23. Oncogenic transformation caused by SV40 and HL-23 are also blocked by either compound. Both compounds exhibit relatively low cytotoxicity at the anti-viral concentrations.

INTRODUCTION

The ability of 3-deazaadenosine (3-deaza-Ado) and 5'-deoxy-5'-isobutylthio-3-deazaadenosine (3-deaza-SIBA) to inhibit growth and replication of RNA viruses have been demonstrated (1, 2). Furthermore, 3-deaza-Ado can inhibit and reverse the oncogenic transformation caused by Rous sarcoma virus (1).

3-Deaza-Ado, an adenosine analog that is not deaminated or phosphorylated, is capable of acting both as a potent inhibitor of S-adenosylhomocysteine hydrolase (AdoHcyase) and as a substrate of this enzyme to yield 3-deaza-AdoHcy. 3-Deaza-Ado has been shown to inhibit methylations in vivo (3-5). Although the equilibrium of the AdoHcyase reaction favors synthesis of AdoHcy, with a Keq of about 1×10^{-6} M, the reaction proceeds physiologically in the direction of hydrolysis due to the enzymatic removal of the products adenosine and homocysteine (Hcy). In vivo inhibition of AdoHcyase by 3-deaza-Ado is probably incomplete,

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ABBREVIATIONS: S-adenosylhomocysteine, AdoHcy; S-3-deazaadenosylhomocysteine, 3-deaza-AdoHcy; S-adenosylhomocysteine hydrolase, AdoHcyase; 5'-deoxy-5'-isobutylthio-3-deazaadenosine, 3-deaza-SIBA; fetal calf serum, FCS; herpes simplex type 1, HSV-1; homocysteine, Hcy; simian virus 40, SV40.

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thus causing an intracellular accumulation of AdoHcy and of 3-deaza-AdoHcy, the latter as a result of the enzymatic condensation of Hcy formed from the hydrolysis of AdoHcy with 3-deaza-Ado (3, 4, 6-8).

As a working hypothesis, we postulated that the intracellular accumulation of S-nucleosidylhomocysteine (NucHcy), i.e. AdoHcy and 3-deaza-AdoHcy, would lead to a selective inhibition of methylation reaction(s) essential to viral replication, such as the methylation of the 5' cap of viral mRNA. We report here that both 3-deaza-Ado and 3-deaza-SIBA exhibit anti-viral activity against 2 DNA viruses, herpes simplex type 1 (HSV-1) and simian virus 40 (SV40), and also against the RNA type C virus, HL-23, isolated from cultured human acute myelogenous leukemia cells (9).

MATERIALS AND METHODS

Cell culture: Cells were cultured in Minimal Essential Medium (MEM) or in RPMI-1640 (GIBCO, Grand Island, N.Y.). Growth medium was supplemented with FCS, 100 units/ml penicillin, and 100 µg/ml streptomycin. All cell counts were done from triplicate plates on a Coulter counter.

Inhibition of plaque formation by herpes simplex type I virus: Culture dishes (60 mm) were seeded with 10^6 mouse L cells each. Twenty-four hours later, the cells were rinsed with phosphate buffered saline and then infected with HSV-1. After 1 hour at 37°, 5 ml of RPMI-1640 medium containing 0.5% methylcellulose, 5% FCS and the appropriate compound were added to each dish. Four days after infection, the infected cells were stained with neutral red and plaques counted.

Inhibition of transformation by SV40: Culture flasks were seeded with 3×10^5 early passage Fisher rat embryo cells, and 24 hours later the cells were infected for 4 hours with diluted tissue culture fluid collected from SV40-infected African Green Monkey kidney cells. RPMI-1640 medium containing 10% FCS and the appropriate compound was then added to the cultures. After treatment for 2 weeks, the infected cells were fixed, stained with Giemsa and the foci counted.

Inhibition of transformation of normal rat kidney cells by HL-23 virus: Culture dishes (60 mm) were seeded with 2×10^5 normal rat kidney cells (NRK 153 Cl 7) in MEM containing 5% FCS, antibiotics and 2 µg/ml of polybrene, a polycation which enhances sarcoma virus infection. Twenty-four hours later, the cells were infected with HL-23 virus for 1 hour at 37°. Culture medium containing the appropriate compound was then added. On day 5 the dishes of infected cells were fixed with methanol, stained with Giemsa and the number of transformed foci counted.

Inhibition of virus production by NRKB/HL-23 cells: For each concentration of compound tested, dishes were seeded with 1×10^5 cells of a clone of normal rat kidney cells (NRKB) which had been transformed by HL-23 virus. Twenty-four hours later, the growth medium was replaced with fresh medium containing the appropriate concentration of compound. After drug treatment for 5 days, the plates were washed 3 times, and the virus collected six hours later was titrated on normal rat kidney cells by counting the foci of transformed cells 5 days after infection.

TABLE I

Anti-viral Activity of 3-Deaza-Ado and 3-Deaza-SIBA on DNA Viruses

Virus	Compounds (μM)		% Inhibition	% Control cell growth
Herpes simplex 1 (plaque formation)	3-Deaza-Ado	25	90	100
		50	95*	100
		100	97*	82
	3-Deaza-SIBA	25	77	94
		50	89*	78
		100	100	63
SV40 (focus formation)	3-Deaza-Ado	10	87	100
		30	100	100
	3-Deaza-SIBA	10	100	100
		30	100	100

*Plaques were small and faint.

RESULTS AND DISCUSSION

Both 3-deaza-Ado and 3-deaza-SIBA markedly affected functioning of both of the DNA viruses used in the present investigation . The replication of HSV-1, as measured by plaque formation, was reduced 90 and 95% by 3-deaza-Ado at 25 and 50 μM, respectively (Table I), while growth of the mouse L cells under these conditions was not affected. 3-Deaza-SIBA caused a 77% reduction in plaque formation at 25 μM, a concentration at which cellular growth was not significantly reduced.

A 100% reduction in the number of oncogenically transformed foci was observed at 30 μM of each compound after infection of early passage Fisher rat embryo cells by SV40. However, cell growth was unaffected during the first 3 days of treatment as measured by cell counts.

Furthermore, both compounds inhibited the induction of oncogenic transformation of normal rat kidney cells caused by the RNA virus HL-23. At 30 μM of either 3-deaza-Ado or 3-deaza-SIBA, foci formation was reduced by 76 and 60%, respectively (Table II). At this concentration, cell growth was not reduced enough to account for the decrease in foci formation (Figure 1). Infectious virus production by the

TABLE II

Effect of 3-Deaza-Ado and 3-Deaza-SIBA on Transformation of Normal Rat Kidney Cells (NRK 153 Cl 7) by HL-23 Virus and on Virus Production by NRKB/HL-23 Cells

Compounds (μM)	% Inhibition foci formation	% Inhibition virus production
3-Deaza-Ado		
11	54	73
30	76	94
100	100	N.D.*
3-Deaza-SIBA		
11	31	89
30	60	92
100	92	N.D.

*N.D., not determined.

transformed cell line, NRKB/HL-23, was also reduced by both compounds. When the production of infectious HL-23 virus was measured for 6 hours after 5 days of treatment of the NRKB/HL-23 cells with 11 μM 3-deaza-Ado or 3-deaza-SIBA, virus

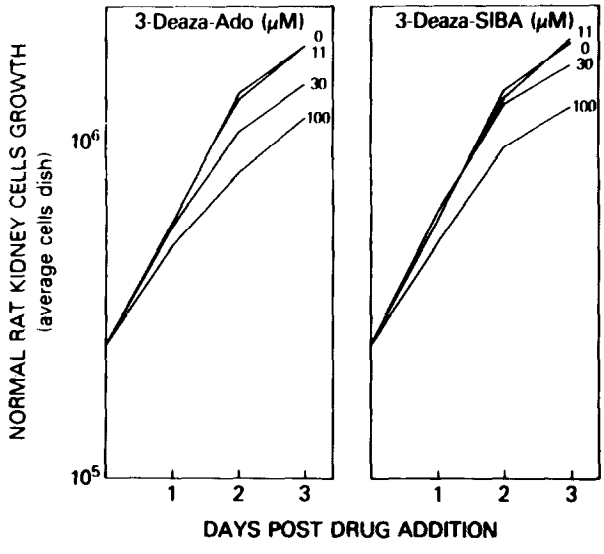

Figure 1. Growth of NRK 153 Cl 7 cells in the presence of 3-deaza-Ado and and 3-deaza-SIBA.

Table III

Failure of 3-Deaza-Ado and 3-Deaza-SIBA to Affect Early Stages of Transformation of Normal Rat Kidney Cells (NRK 153 Cl 7) by HL-23 Virus*

	Duration of treatment (hours)			
Compounds (μM)	0-4	0-8	8-24	8-96
	% Control foci			
3-Deaza-Ado				
15	100	90	90	20
30	100	80	80	10
3-Deaza-SIBA				
15	100	90	100	30
30	100	90	100	20

*Cells were infected and treated as in Methods

production per cell was reduced by 73 and 89%, respectively (Table II). It has been shown that prolonged treatment of chick embryo cells transformed by Rous sarcoma virus with 3-deaza-Ado inhibited some of the biochemical and morphological characteristics typical of oncogenic transformation (1). In contrast, prolonged treatment of NRKB/HL-23 cells with 3-deaza-Ado did not result in any changes in the morphology of the transformed cells or in their ability to grow in suspension.

As reported earlier (1), the inhibitory effect of 3-deaza-Ado on focus formation depends on the timing of treatment of cells after infection. Treatment of the infected cells with either 3-deaza-Ado or 3-deaza-SIBA for durations less than 24 hours, during which period presumably DNA synthesis via reverse transcriptase takes place, had no effect on subsequent transformation (Table III). However, striking inhibition of focus formation was observed if either compound was

added 8 hours post-infection and left in the culture medium for the duration of the 96 hours required for focus formation.

The present investigations extend earlier reports and indicate that both 3-deaza-Ado and 3-deaza-SIBA not only inhibit the growth and replication of several RNA viruses, but also affect DNA viruses, namely growth of HSV-1 and transformation by SV40. Although the anti-viral activity of 3-deaza-Ado is probably due to a perturbation of the ratio of AdoMet/NucHcy, leading subsequently to the inhibition of methylation(s) (1, 3, 4), the biochemical mode of action of 3-deaza-SIBA is not clear.

Both 3-deaza-SIBA and its parent analog, 5'-deoxy-5'-isobutylthio-adenosine (SIBA), may be viewed as analogs of either AdoHcy or methylthioadenosine (10, 11). In vitro they exhibit only weak inhibitory activity toward AdoHcyase (2) and methyltransferases (12, 13). It has been postulated that SIBA inhibits the methylation of the 5' cap of viral mRNA (12) and tRNA (13). Recently it has been shown that SIBA can also inhibit glucose transport into cells (14). Further investigations are therefore needed to clarify the precise mode of action involved in the antiviral activity of these 2 compounds.

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