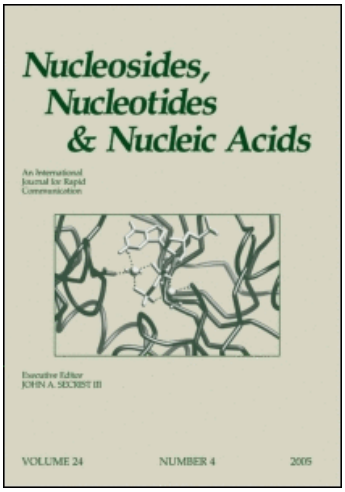

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6-AZACYTIDINE - COMPOUND WITH WIDE SPECTRUM OF ANTIVIRAL ACTIVITY

I. Alexeeva[a]; N. Dyachenko[b]; L. Nosach[b]; V. Zhovnovataya[b]; S. Rybalko[c]; R. Lozitskaya[d]; A. Fedchuk[e]; V. Lozitsky[e]; T. Gridina[e]; A. Shalamay[a]; L. Palchikovskaja[a]; O. Povnitsa[b]
[a] Institute of Molecular Biology and Genetics, Kyiv, Ukraine [b] Institute of Microbiology and Virology, Ukraine [c] Institute of Epidemiology and Infectious Diseases, Kyiv, Ukraine [d] Physical-Chemical Institute, Odesa, Ukraine [e] Institute of Emerging Infectious Diseases, Odesa, Ukraine

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6-AZACYTIDINE - COMPOUND WITH WIDE SPECTRUM OF ANTIVIRAL ACTIVITY

I. Alexeeva,[1] N. Dyachenko,[2,*] L. Nosach,[2] V. Zhovnovataya,[2] S. Rybalko,[3] R. Lozitskaya,[4] A. Fedchuk,[5] V. Lozitsky,[5] T. Gridina,[5] A. Shalamay,[1] L. Palchikovskaja,[1] and O. Povnitsa[2]

[1]Institute of Molecular Biology and Genetics, Kyiv, Ukraine
[2]Institute of Microbiology and Virology, Kyiv-143, Ukraine 03143
[3]Institute of Epidemiology and Infectious Diseases, Kyiv, Ukraine
[4]Physical-Chemical Institute, Odesa, Ukraine
[5]Institute of Emerging Infectious Diseases, Odesa, Ukraine

ABSTRACT

6-azacytidine demonstrates activity against adenoviruses types 1, 2, 5. It inhibit synthesis of viral DNA and proteins. 6-AC shows antiherpetic and antiinfluenza action during experimental infection in mice. 6-AC is prospective for drug development as an antiviral substance with a wide spectrum of activity.

INTRODUCTION

The chemotherapy of virus infections is developing rather intensively now. It is very important for medicine owing to the wide spread of influenza virus, herpes simplex virus and adenoviruses and their important role in pathology. Modified nucleosides have proven to be the useful substances for antiviral and anticancer drug design. 6-Azacytidine (2-β-D-ribofuranosyl-5-amino-1,2,4-triazin-3(2H)-on; 6-AC) is an original structural cytidine analogue (Fig. 1). The objective of the present investigation is to study of the antiviral activity 6-AC in different *in vitro* and *in vivo* models.

*Corresponding author. Fax at +38 044 266 23 79; E-mail: dyachenko@srv.imv.kiev.ua

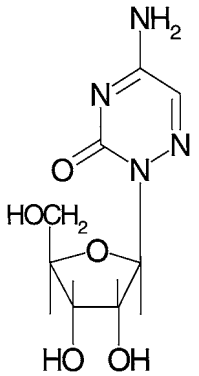

Figure 1. 6-Azacytidine.

MATERIALS AND METHODS

The method of synthesis for 6-azacytidine, based on a simplified one-step variant of a ≪silyl condensation≫, was developed in our laboratory. Selective N_2-glycosylation of 5-amino-1,2,4-triazine-3(2H)-one (6-azacytosine) by peracylribofuranose in the presence of hexamethyldisilazane, trimethylchlorosilane, and tin tetrachloride (catalytic) gives the tri-o-acyl derivative of 6-azacytidine in 68% yield. The subsequent unblocking of the glycon moiety in alkaline medium led to unprotected 6-AC (1). The structure of 6-AC was confirmed by their ^{1}H-NMR spectral data.

After crystallization from 80% ethanol 6-AC was obtained as colorless crystals, m.p. 223–225°C. UV(H_2O): λ_{max}264 nm (lg ε 3.9). ^{1}H-NMR (DMSO-d_6) δ: 8.018 (s, 1H, H^a-N); 7.901 (s, 1H, H^b-N); 7.503 (s, 1H, H-C_5); 5.970 (d, 1H, H-1′); 5.191 (d, 1H, OH-2′); 4.978 (d, 1H, OH-3′); 4.680 (t, 1H, OH-5′); 4.200 (dd, 1H, H-2′); 3.950 (dd, 1H, H-3′); 3.486 (m, 1H, H^a-5′); 3.359 (m, 1H, H^b-5′).

Hep-2 or HeLa cells were grown in tubes with the strips of the cover glasses. After 48 h, cells were infected with adenoviruses type 1, 2, 5 (Ad-1, Ad-2 or Ad-5), further, after 60#min adsorption of virus, cells were washed with Hanks'

Table 1. Influence of 6-AC on the Ad 2 Reproduction in Hep-2 Cell Culture

Concentration μg/ml	Inhibition of Number of Cells with Intranuclear Inclusions, %	Virus Titres lg CTD_{50}
125	100	0
62	100	0
16	82	2.7
8	86	—
0.5	62	2.7
0 (Control)	—	4.45

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Table 2. Influence of 6-AC on the Ad 5 Reproduction in Hep-2 Cell Culture

Concentration of 6-AC, μg/ml	Percent of Cells with Viral Inclusion Bodies	Inhibition of Infection, %
125	0	100
62	0	100
16	0	100
8	0	100
0.5	24	61
0 (Control)	62	

solution and incubated in Eagle medium carrying 6-AC of varying concentrations. 48 h following infection the cells were fixed, stained with 0.01% acridine orange solution and investigated by luminescent microscopy. The infected cells were revealed by presence of the virus-specific intranuclear inclusions. An inhibition of

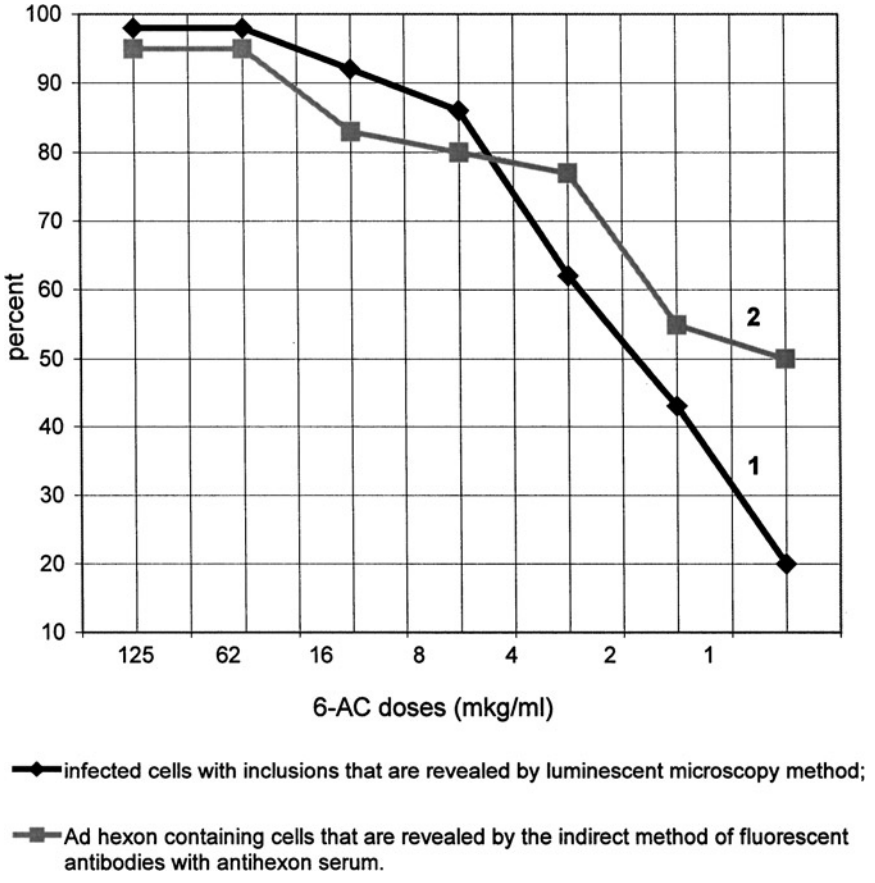

Figure 2. The inhibitory action of 6-AC on quantity of ad-1 infected cells with intranuclear inclusion (1) and hexon antigen (2) in cell culture HeLa.

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Table 3. Prevention Action of 6-AC During Experimental Herpetic Infection

Preparation	Doses mg/kg	Quantity of Mice in Experiment	Lethal Cases		MP**	IE**
			Total	%		
6-AC	0.01	14	8	57.1	1.73	42.19
	0.1	14	12	85.7	1.16	13.79
	0.5	14	10	71.4	1.40	28.57
	1.0	12	2	16.6	6.02	83.38
Acyclovir	1.0	14	4	28.5	3.50	71.14
Placebo		14	14	100		

*-Multiple of protection.
**-Index of efficiency.

virus reproduction in cell cultures by 6-AC was determined by a reduction in the number (percent) of cells with inclusions (1).

Mice infected intracerebrally with herpes simplex virus (HSV) type 1 were injected intramusculary with 6-AC in 24 hrs before or after infection.

Influenza infection in mice (12–14 g) was modeled by intranasal challenge of A/PR/8/34 (H1N1) strain using 4 animals per each logarithmic dilution of virus containing material. 6-AC was instillated intranasally in dose 25 mg/kg per day during 5 days beginning from 24 hours before infection. The death of animals was registered during 14 days after infection.

RESULTS

6-AC over a wide range of concentrations (0,5–125 μg/ml) inhibits the synthesis of infective Ad, formation of intranuclear inclusion bodies and hexon antigen (Tables 1 and 2, Fig. 2). It also inhibits the synthesis of viral DNA and viral polypeptides (2, 3). 6-AC in some high concentrations completely caused the switch-off of adenovirus genome expression.

The selectivity index of 6-AC was 62–125 towards Ad 1 and Ad 2 and 250 towards Ad 5. 6-AC was effective on the model of herpetic meningoencephalitis

Table 4. Therapeutic Action of 6-AC During Experimental Herpetic Infection

Preparation	Doses mg/kg	Quantity of Mice in Experiment	Lethal Cases		MP	IE
			Total	%		
6-AC	0.01	14	10	71.4	1.40	28.57
	0.1	14	10	71.4	1.40	28.57
	0.5	14	4	28.5	3.50	71.40
Acyclovir	1.0	14	6	42.8	2.30	56.59
Placebo		14	14	100		

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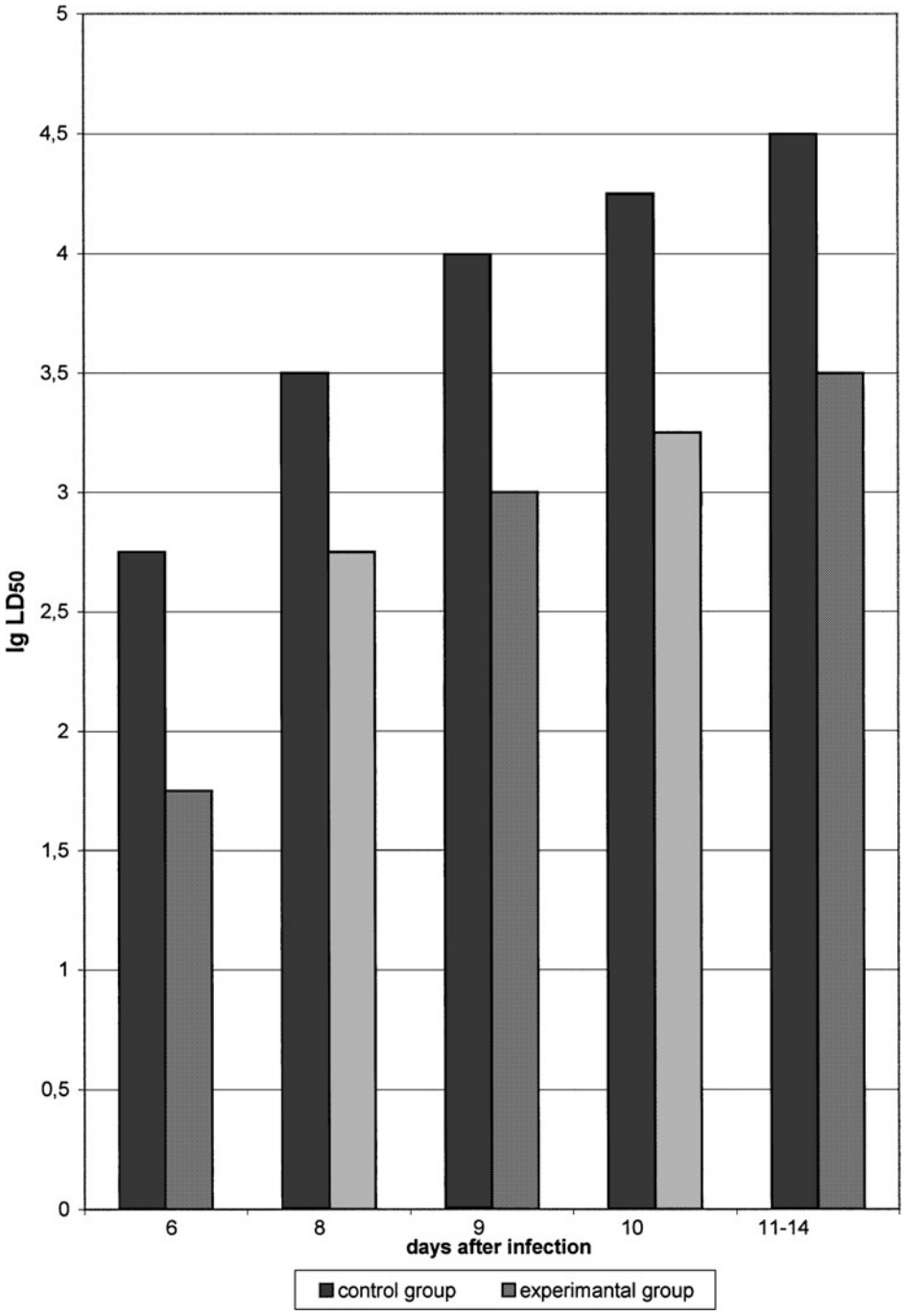

Figure 3. Efficacy of 6-AC during experimental influenza infection in mice.

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in mice. It was more active than reference compound acyclovir ("KRKA", Slovenia) if preparations were used on preventive or therapeutic schemes (Tables 3 and 4).

The intranasal instillations of 6-AC decreased lethality reliably during experimental influenza infection in mice (Fig. 3). The difference between control and experimental groups was 1,0 lg LD_{50}.

So, 6-AC has a wide spectrum of antiviral activity. It is a prospective substance for drug development and for synthesis of its derivatives.

6-AC could be used as the reference antiviral preparation to be compared with its derivatives containing macrocyclic pyridinophanes [4], which are under investigation in our group through INTAS-grant 97-31528.

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