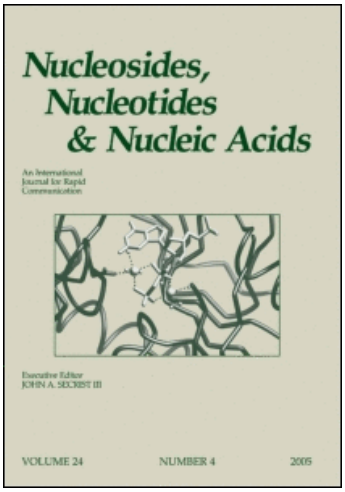

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Inhibition of Human Immunodeficiency Virus (HIV) Production by 5'-Hydrogenphosphonates of 3'-Azido-2',3'- dideoxynucleosides

Natalya B. Tarussqva[a]; Marina K. Kukhanova[a]; Alexander A. Krayevsky[a]; Edward K. Karamov[b]; Vladimir V. Lukashov[b]; Galina B. Kornilayeva[b]; Mariana A. Rodina[b]; Georgii A. Galecov[b]
[a] V. Engelhardt Institute of Molecular Biology, the USSR Academy of Sciences, Moscow, USSR [b] Ivanovskii Institute of Virology, the USSR Academy of Medical Sciences, Moscow, USSR

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INHIBITION OF HUMAN IMMUNODEFICIENCY VIRUS (HIV) PRODUCTION BY 5'-HYDROGENPHOSPHONATES OF 3'-AZIDO-2',3'-DIDEOXYNUCLEOSIDES

Natalya B.TARUSSOVA, Marina K.KUKHANOVA, Alexander A.KRAYEVSKY, Edward K.KARAMOV*, Vladimir V.LUKASHOV*, Galina B.KORNILAYEVA*, Mariana A.RODINA*, Georgii A.GALEGOV

V.Engelhardt Institute of Molecular Biology, the USSR Academy of Sciences, Vavilov str.32, Moscow 117984, USSR; *D.Ivanovskii Institute of Virology, the USSR Academy of Medical Sciences, Gamaleya str.16, Moscow 123098, USSR

ABSTRACT. 3'-Azido-2',3'-dideoxynucleoside 5'-hydrogenphosphonates with thymine, adenine, guanine and cytosine bases inhibit HIV-1 reproduction in MT4 cell cultures. The most active was 5'-hydrogenphosphonate of 3'-azido-2',3'-dideoxythymidine. Equimolar mixture of all four hydrogenphosphonates is shown to be less toxic in comparison with each of compounds taken separately.

Among 3'-azido-2',3'-dideoxynucleosides a high anti-HIV activity has been found for 3'-azido-2',3'-dideoxythymidine (AZT), whereas 3'-azido-2',3'-dideoxynucleosides with adenine (AZA), guanine (AZG) and cytosine (AZC) bases are shown to be less active [1,2]. The reasons for such difference in activity depend mainly on poor phosphorylation of the latter three compounds into their 5'-triphosphates in cells as compared with AZT. At the same time 5'-triphosphates of all 3'-azido-2',3'-dideoxynucleosides blocked viral reverse transcriptases activity in nearly the same degree [3,4].

The main toxic effect of AZT on human is connected with bone marrow supression [5]. It probably occurs due to AZT dependent disbalance of natural pool of 2'-deoxynucleoside triphosphates

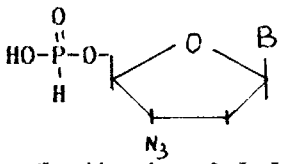
Table 1. Anti-HIV activity and cytotoxicity of hydrogenphosphonates I-IV

Compound	ED_{50}, μM	CD_{50}, μM	SI
AZT	0.06	72	1200
I	0.03	102	3400
II	0.3	104	347
III	0.9	140	156
IV	>1	104	<104
mixture I-IV	0.09	250	2778

ED_{50} - fifty percent effective dose, decreasing reverse transcriptase activity by 50%; CD_{50} - fifty percent cytotoxic dose, based on the reduction of the viability of noninfected cells. SI - selectivity index - ratio CD_{50} to ED_{50}.

in cells [6]; this disbalance induces depression of DNA replication in proliferating cells [7].

As it was reported by us earlier, three types of 5'-phosphonates of 3'-azido-2',3'-dideoxynucleosides [8,9] and some other modified nucleosides[10] blocked effectively the production of HIV-1 in cell cultures. These investigations were continued in the HIV-1 infected MT4 cells.



I B = Thy
II Ade
III Gua
IV Cyt

Synthesis of I-IV was made as in [8,9]. The action of phosphonates I-IV on HIV reproduction was evaluated on the 4th and 7th day of incubation by measuring reverse transcriptase activity, immunoenzyme analysis [8], reclastering method [10] and counting alive cells.

Table 1 shows that all four phosphonates I-IV block the HIV-1 reproduction, the selectivity index of I-IV as a rule is higher than that of the corresponding nucleosides. It should be noted specially that the toxicity of the I-IV mixture was 2-3 fold lower as compared with that for phosphonates I-IV separately. We beleive that this fact can be interpreted as decreasing of the disbalance of the natural 2'-deoxynucleoside 5'-triphosphate *pool in cells*.

Table 2. Protection of HIV-infected cells with phosphonates I-IV by reclustaring method and cytotoxic effect of I-IV on noninfected cells

Compound and its concentration, μM		Clusters formation* after incubation during 4 days	7 days	Survival cells after 7 days incubation with substances %
AZT	1	+++	-	71
	10	++	-	73
I	1	+++	+-	80
	10	+++	+-	75
II	1	++	-	72
	10	++	-	70
III	1	+++	-	69
	10	+++	-	68
IV	1	+-	-	68
	10	++	-	69
mixture I-IV	0.25 each	+++	+	72
	2.5	+++	+	81
-		-	-	19
mock-infected cells (control)		+++	+++	96

* - no clusters; +- a few clusters contain less than 20 cells in each; + clusters contain more than 20 cells; ++ clusters contain more than 60 cells; +++ all the cells in clusters

Table 3. Anti-HIV-1 activity of AZT and I

Substances	ED_{50}, μM	CD_{50}, μM	SI	Cell culture	Reference
AZT	0.005	210	30800	MT-4	[11]
	0.0139*	154	11079*		
I	0.072	2500	34700		
	0.2763*	2500	9050*		
AZT	0.02	>200		A301	J.A.H.Verheiden Personal communication
I	0.017	>200			

All designations as in Table 1. Activity of substances was tested by immunoenzyme analysis and * reverse transcriptase test.

Analysis of the above mentioned data shows that all hydrogenphosphonates inhibit the HIV reproduction in MT4 cells and the equimolar mixture of I-IV is less toxic as compared with each of I-IV taken separately.

According to the immunoenzyme analysis the activity of the I-IV mixture was higher as compared with each of I-IV (data not shown).

Table 2 demonstrates the results of determination of the I-IV activity by reclustering method. Clusters of infected cells during incubation with virus dissociated. Each compound I-IV protected clusters due to HIV reproduction inhibition. As one can see from Table 2, after 4 days of incubation AZT and I-IV protected cell clusters comparatively well, but after 7 days incubation a minor protection was registrated only for I. In contrast, the mixture of I-IV protects more than 20% of cells in clusters. This fact illustrates higher efficiency of the I-IV mixture as compared with I-IV taken separately.

Activity of I synthesised by us was tested in some other laboratories. Table 3 summerizes these data.

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