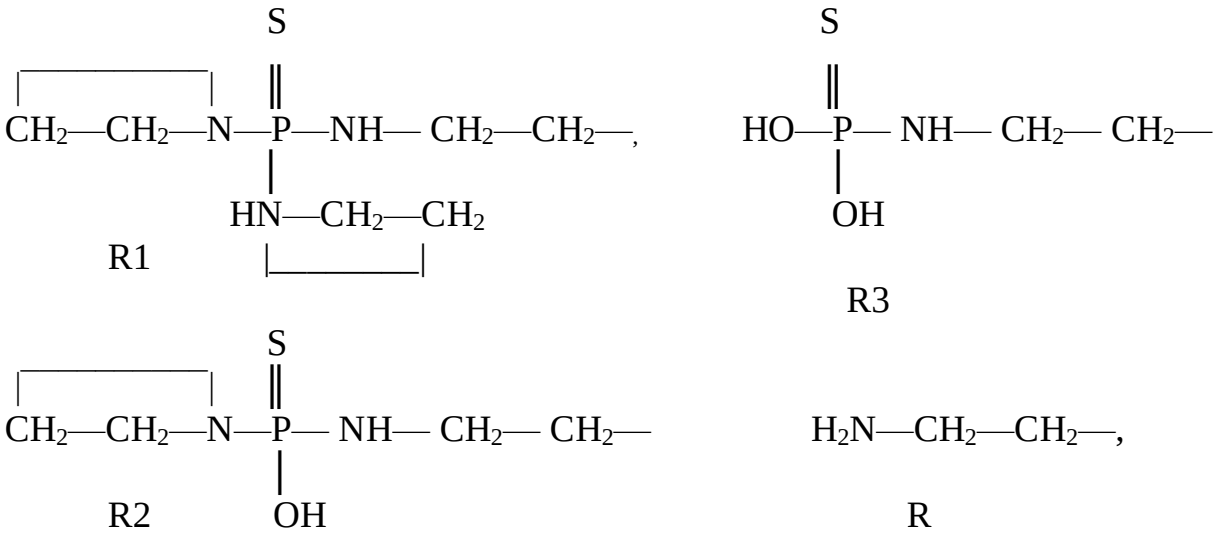
The obtaining of antitumor preparations from alkylated nucleic acids and their monomeric components

T.P. Voloshchuk, Yu.V. Patskovsky, A.I. Potopalsky

Institute of Molecular Biology and Genetics of NAS of Ukraine, Kyiv, Ukraine
e-mail:t.p.voloshchuk@imbg.org.ua

Nucleic acids (NA) and their several components (bases, nucleosides and nucleotides), being initially biologically active substances, after their alkylation with substances possessing antitumor action, obtain another antitumor effect, but practically without mutagenicity that is inherent to alkylating agent. Thus, in the number of our works, devoted to NA components alkylation with different agents [1-3], it was determined that molecules, modified with polyfunctioning alkylating agents, depending on the conditions of reaction's carrying out, may contain different alkyl radicals. This fact concerns, first of all, trifunctional alkylating agent thioteph (N',N'',N''' – triethylenethiophosphoramide, thiophosphamide) that is able to give four types of alkyl radicals as sequential aziridine cycles breakdown (Scheme).



The molecules, modified by R1 radical, have the highest antitumor activity, and the lowest – R radicals [4]. This can be explained by the fact that preparations with R1 radical are not only alkylated as the preparations with R radical, but also

themselves have property to alkylate i.e., likewise thioteph, are the alkylating agents.

From the table given below, it is seen that thioteph, until now used in clinics as an antitumor preparation, has lower effectiveness than alkylated DNA (DNT) and also than its several components.

In the experiment on Ehrlich's ascites tumor (EAT) cells of the mice *in vitro* it was shown that DNA and especially its guanine nucleotide (GMP) are the stimulants of the tumor growth, whereas DNT inhibits the growth of tumor cells more than even antitumor preparation thioteph (Table).

On the adenine example, it is also shown that not so much the fact of alkylation is as of an importance, rather than the place in basis molecule, where the reaction took place. The most active preparations among investigated ones are the adenine basis and nucleoside, modified by nitrogen in N1 thiotheph position with one open aziridine cycle (radical R1). N1-substituted guanosine nucleoside and nucleotide with R1 radical have rather less activity (besides, comparable to thioteph antitumor activity, but without its mutagenic effect).

Antitumor action of the thioteph alkylated DNA preparations of different derivation (cattle spleen, salmon sperm, chicken erythrocytes), yeast RNA and mononucleotides – ATP and GTP proved in the experiment *in vivo* on the mice with transplantable Ehrlich's carcinoma. Therapeutic doses (100 – 300 mg/kg of weight) of the mentioned preparations provide inhibition of tumors growth on 90-100% at toxic LD 50 up to 2 g/kg.

Influence of the biologically active preparations on labeled nucleosides inclusion in NA of EAT cells in vitro*

Preparations	Nucleosides inclusion in % comparing to control	
	^{3}H-thymidine	^{3}H-uridine
EAT DNA (50 mkg/ml)	105.1 ± 11.7	102.0 ± 7.3
EAT DNT (50 mkg/ml)	46.0 ± 4.0	70.7 ± 21.1
Thioteph (1.5 mM) mkg/ml)	57.0 ± 16.4	80.1 ± 3.7
1-R-adenine	97.1 ± 3.6	87.4 ± 9.6
1-R1-adenine	39.4 ± 4.2	78.6 ± 11.7
3-R-adenine	96.3 ± 4.5	92.5 ± 5.7
3-R1-adenine	56.4 ± 7.7	83.4 ± 6.5
9-R-adenine	93.6 ± 4.8	98.6 ± 8.4
9-R1-adenine	74.0 ± 4.9	101.8 ± 9.1
1-R1-adenosine	37.3 ± 10.7	55.7 ± 7.8
AMP	96.0 ± 1.3	48.6 ± 5.2
1-R1-AMP	76.5 ± 4.6	101.5 ± 8.5
Guanosine	84.0 ± 6.3	118.0 ± 4.3
7-R1-guanosine	52.2 ± 6.8	86.6 ± 7.3
GMP	203.5 ± 15.7	123.0 ± 16.5
7-R1-GMP	57.9 ± 4.7	74.5 ± 3.2

*1. The time of cells incubation with DNA, DNT, thioteph and other preparations (the upper part of the table) – is 30 min, with NA alkylated monomeric components – 90 min.

2. The time of incubation with labeled nucleosides: thymidine – is 105 min, uridine – 20 min. 3. R – aminoethyl, R1 – phosphaminoethyl radicals (scheme). 4. Concentration of the preparations (except for mentioned in the table) – 1 mM.

Alkylated DNA introduction to the animals on the early tumor development stages (usually on the 4th day) brings to tumor growth inhibition, cells differentiation and apoptosis activation. The introduction on the later stages (for instance, on the 8th

day) is not effective, as it is accompanied by intensive tumor cells death that leads to high intoxication of the animals and to their death.

Thus, the mentioned data show availability of use the alkylated DNA preparations of different derivation, modified by multifunctional alkylating agents, in particular – thioteph as antitumor drugs.

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