THE PROBLEM OF CORRELATION BETWEEN THE ANTIPHAGE AND ANTIMITOTIC EFFECTS OF ALKYLATING COMPOUNDS

(UDC 615.771.7-092.18)

L. B. Borisov, G. S. Yakovleva, and S. V. Livshina

Chair of Microbiology, Leningrad Medical Institute of Sanitation and Hygiene
(Presented by Member of the Academy of Medical Sciences USSR, V. D. Timkov)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 60, No. 12,
pp. 90-94, December, 1965
Original article submitted April 13, 1964

It is known that alkylating agents from the group of di-β-chloroethylamine and ethyleneimine possess pronounced antiphage and antimitotic activity [1-8]. It was recently demonstrated [4] that of the 18 investigated chloroethylamino derivatives exerting antitumoral action, 14 were characterized by selective antiphage activity. However, it is not yet clear to what degree the antiphage action of the alkylating substances corresponds to their antimitotic properties. The solution of this problem, in our opinion, would be of definite interest in the use of a phage model for preliminary selection of potential antitumoral agents among alkylating compounds.

In this work, we endeavored to give a comparative quantitative evaluation of the antiphage and antimitotic actions of chloroethylamino- and ethyleneimino- compounds used as official antitumoral remedies.

EXPERIMENTAL PROCEDURE

In the experiments, we used phages T1, T7, and coli-phage 026, which showed the highest sensitivity to alkylating substances in our previous investigations [1,2]. For an objective quantitative evaluation of the antiphage activity of the investigated compounds, the latter was expressed in inhibitor doses (ID/50, dose of the substance inactivating 50% of the test phage particles in a definite segment of time at 37°). Physiological solution (pH 6.1) was used as the solvent for the dichloroethylamines, and acetate buffer (pH 5.0) for the ethyleneimines, since in other buffer solutions the activity of the investigated compounds was appreciably reduced [2].

The original amount of the test phage was 10^7 particles per ml in all experiments. The investigation procedure was described earlier [1,2].

The antimitotic activity of the alkylating substances was studied in vitro with respect to HeLa and D-6 tumor cells in tissue culture. For this purpose, the solutions of the alkylating agents were introduced into synthetic medium No. 199 with 20% horse blood serum or without it. When the pH of the medium changed, it was acidified with CO_2 or alkalized with a 1.4% soda solution until the original pH 7.0-7.2 was reached. The corresponding solutions of each preparation in nutrient medium were introduced into test tubes with tissue culture containing 300 thousand ± 10,000 cells per ml of medium. Thereupon, the original nutrient medium was preliminarily removed from the test tubes. In all the experiments, we used three test tubes per dose of each preparation. Further incubation was conducted for 24 h. The antimitotic action of the alkylating substances was evaluated according to the ability of the culture to grow in the second generation. For this purpose, the precipitate of cells, washed free of the preparation by centrifuging, was placed in fresh nutrient medium and incubated. The absence of growth was observed only in the case of death of approximately 75% of the cells. This dose was denoted as the least inhibitory dose. The experimental results were considered only in the case when there was no degeneration of the cells in control test tubes, containing an equal volume of solvent in place of the solution of the preparation, and full-valued growth of the tissue culture was observed in reinocula.

TABLE 1. Dependence of ID/50 of Dichloroethylamines on the Type of Test Phage

Preparation	Concentration (in M)	Test phage*		
		O 26	T1	T7
Novembichin	2×10^{-4}	99,98	97,0	99,99
	1×10^{-4}	98,4	**66,0**	99,96
	5×10^{-5}	81,0	35,0	97,0
	$2,5 \times 10^{-5}$	**51,0**	18,6	**58,0**
	$1,2 \times 10^{-5}$	27,5	0	18,8
Sarcolysine	$2,5 \times 10^{-3}$	94,1	98,5	97,5
	$1,2 \times 10^{-3}$	**50,0**	**49,6**	**46,9**
Dopan	$1,2 \times 10^{-3}$	82,0	95,0	89,0
	5×10^{-4}	**54,0**	**60,0**	**53,2**
	$2,5 \times 10^{-4}$	30,6	31,9	25,7
Degranol	$1,2 \times 10^{-3}$	0	99,0	99,8
	5×10^{-4}	0	79,0	68,0
	$2,5 \times 10^{-4}$	0	**51,0**	**50,0**

*Number of inactivated phage particles (in % of control) after treatment with alkylating agent.

Note: Here and in Table 2, the underlined numbers indicate the percent of phage particles inactivated by the dose of the preparation taken as ID/50.

TABLE 2. Determination of ID/50 for Ethyleneimino-Derivatives

Preparation	No. of inactivated phage particles (in % of control) after treatment with solutions of ethyleneimines (in M)			
	$1,25 \times 10^{-3}$	$6,2 \times 10^{-4}$	$3,4 \times 10^{-4}$	$1,5 \times 10^{-4}$
Ethoxene	99,99	97,7	67,0	**59,0**
MEI-2*	99,99	96,2	**60,0**	26,0
Thiophosphoramide	99,0	92,2	**75,0**	0
Dipin	98,8	92,0	**57,0**	0

*MEI-2 (N,N'-malonyl-bis-ethyleneimine) was synthesized by L. B. Dashkevich and V. G. Beilin. The antiphage activity of this preparation was described in our previous work [2].

TABLE 3. Inhibitory Effect of Alkylating Substances with Respect to HeLa and D-6 Cells in Tissue Culture

Preparation	Tissue culture	Growth of cells in presence of alkylating agents in concentration (in M)								
		$1{,}5 \times 10^{-3}$	$7{,}5 \times 10^{-4}$	$3{,}8 \times 10^{-4}$	$2{,}5 \times 10^{-4}$	$1{,}9 \times 10^{-4}$	$1{,}0 \times 10^{-4}$	$5{,}0 \times 10^{-5}$	$2{,}5 \times 10^{-5}$	$1{,}0 \times 10^{-5}$
Novembichin	HeLa	—	—	—	—	—	—	+	+	+
	D-6	—	—	—	—	—	—	—	+	+
Sarcolysine	HeLa	—	—	—	—	—	+	+	+	+
	D-6	—	—	—	—	—	—	+	+	+
Dopan	HeLa	—	—	—	—	—	+	+	+	+
	D-6	—	—	—	—	—	—	+	+	+
Degranol	HeLa	—	—	—	—	—	—	—	—	+
	D-6	—	—	—	—	—	—	—	—	+
Ethoxine	HeLa	—	—	—	—	+	+	+	+	+
	D-6	—	—	—	—	—	—	Not determined		
N,N'-Malonyl-bis-ethyleneimine/MEI-2	HeLa	—	—	—	—	+	+	+	+	+
	D-6	—	—	—	—	—	—	Not determined		
Thiophosphamide	HeLa	—	+	+	+	+	+	+	+	+
	D-6	—	—	—	+	+	+	+	+	+
Dipin	HeLa	—	—	—	+	+	+	+	+	+
	D-6	—	—	—	—	—	+	+	+	+

TABLE 4. Relationship between Antiphage and Antimitotic Effects of Alkylating Compounds

Preparation	Inhibitory dose of preparation (in M) with respect to cells		Antiphage dose of preparation (ID/50)
	HeLa	*D-6*	
Dichloroethylamines:			
Novembichin	$1,0 \times 10^{-4}$	5×10^{-5}	1×10^{-4}
Sarcolysine	$1,9 \times 10^{-4}$	1×10^{-4}	$1,2 \times 10^{-3}$
Dopan	$1,9 \times 10^{-4}$	1×10^{-4}	5×10^{-4}
Degranol	$2,5 \times 10^{-5}$	$2,5 \times 10^{-5}$	$2,5 \times 10^{-4}$
Ethyleneimines:			
Ethoxine	$2,5 \times 10^{-4}$	1×10^{-4}	$1,5 \times 10^{-4}$
MEI-2	$2,5 \times 10^{-4}$	1×10^{-4}	3×10^{-4}
Thiophosphamide	$1,5 \times 10^{-3}$	$3,8 \times 10^{-4}$	3×10^{-4}
Dipin	$3,8 \times 10^{-4}$	$1,9 \times 10^{-4}$	3×10^{-4}

EXPERIMENTAL RESULTS

The inhibitory dose, determined for dichloroethylamines, depended on the type of test phage used. Thus, ID/50 for novembichin was somewhat less than for sarcolysine and dopan. In the experiments with degranol, this value could be established only according to phages T1 or T7, since the coli phage 026 proved comparatively resistant to the latter compound (Table 1).

The data presented in Table 1 were obtained in two-minute treatment of the test phages with novembichin, sarcolysine, and degranol. In the experiments with dopan, the duration of treatment of the phages was increased to an h, since in the case of a shorter exposure, inactivation of the test phages was extremely weak.

ID/50 for ethyleneimino-derivatives was established according to T1 phage in a 30-minute exposure; the indicated value was approximately the same for all the investigated substances (Table 2).

The antimitotic action of the alkylating agents depended on the composition of the nutrient medium, on the strain of cells, and on the concentration of the preparation. The experimental results indicated that in medium No. 199 with 20% blood serum, the activity of the alkylating agents was lowered two to four-fold, depending on the chemical structure of the preparation. This may be explained by the different ability of the investigated compounds to react with the blood serum proteins. Hence, our further investigations were conducted in serum-free medium.

A comparative evaluation of the inhibitory activity of alkylating agents with respect to HeLa and D-6 cells (Table 3) indicated that the latter proved more sensitive to them. Moreover, dichloroethylamines (novembichin, sarcolysine, dopan, degranol) exerted a stronger inhibitory action than the ethyleneimines (ethoxine, MEI-2, thiophosphamide, dipin).

The data obtained permitted us to compare the inhibitory doses of each of the investigated substances with respect to both strains of tumor cells and the test phages (Table 4). In this case, it was found that the inhibitory doses of chloroethylamines for tumor cells were lower than for bacteriophages. However, in spite of the difference noted, the chloroethylamines were arranged in antimitotic activity in the same order in which they induced inactivation of the test phages, with the exception of degranol. The latter proved to be a stronger inhibitor of tumor cells in comparison with novembichin, although its antiphage activity was less pronounced.

In experiments with ethyleneimines, it was established that their antimitotic doses in many cases almost entirely coincided with ID/50, established for test phages.

Of course, it would be difficult to expect that the inhibitory doses of alkylating substances, established for test phages and cells, would entirely coincide in all cases, all the more in that each of these substances exerted a destructive action on the different strains of tumor cells in different concentrations. However, the correspondence in the degree of inhibitory action on the phages and cells, detected in almost all the compounds studied, will permit the use of these phages for preliminary evaluations of new alkylating agents with unknown biological activity.

LITERATURE CITED

1. L. B. Borisov, Mikrobiologiya, 38, 1-2, 61 (1964).
2. L. B. Borisov, Byull. Éksper. Biol., 3, 78 (1964).
3. D. M. Gol'dfarb and T. N. Broker, Vopr. Virusol., 1, 103 (1959).
4. V. D. Timakov, D. M. Gol'dfarb, Yu. K. Fomichev, et al., Vopr. Virusol, 6, 650 (1963).
5. E. A. Timofeevskaya, in the book: Problems of the Etiology and Pathogenesis of Tumors [in Russian], Moscow (1957), p. 75.
6. E. A. Timofeevskaya, in the book: Problems of the Etiology and Pathogenesis of Tumors [in Russian], Moscow (1957), p. 79.
7. T. G. Khaleeva, Byull. Éksper. Biol., 1, 95 (1960).
8. A. Loveless, Colloq. internat. Centre nat. rech. scient., 88, 261 (1960).