INHIBITORS OF AN RNA-DEPENDENT DNA POLYMERASE

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1. Introduction

Much interest of late has centered around RNA-directed DNA synthesis. Initially, with the finding of an RNA-dependent DNA polymerase in oncogenic RNA viruses [1–5] and what was described as the exclusive presence of the polymerase in acute leukemia cells as opposed to 'normal' cells [6], speculation as to a molecular basis for leukemogenesis and a predictive test for leukemia abounded. However, with the identification [7, 8] of the polymerase in 'normal' cells (cells not known to be RNA virally transformed) the hope of an absolute predictive index for leukemia or neoplasia has lost credibility, and the RNA-dependent DNA polymerase may be of more interest from the standpoint of gene amplification as opposed to a neoplastic predictor. In our studies of this polymerase we have undertaken an extensive, systematic experiment designed to note the effects of a number of potential inhibitors (primarily antibiotics) on the RNA-dependent DNA polymerase in vitro. The present brief report gives the results of these experiments. Several compounds were shown to be potent inhibitors of an RNA-dependent DNA from mouse leukemia cells.

2. Materials and methods

Two cell lines were utilized, L5178Y cells grown in suspension culture and L1210 cells harvested from CDF_1 mice. Details of cell culture and harvest are given in previous publications [9–11]. All cells were used in logarithmic growth. A 'nucleic acid free extract' of the L5178Y cells was prepared by the method of Gallo et al. [6] as described previously [8]. Exponential L5178Y cells were vigorously homogenized for 30 strokes in a Potter Elvehjem homogenizer at 0°. The homogenizing solution was 5 volumes of 25 mM tris-HCl buffer pH 8.3, 1 mM $MgCl_2$, 6 mM NaCl, 5 mM dithiothreitol and 0.15 mM EDTA. The samples were centrifuged at 27,000 *g* and the pellet discarded. Nucleic acids were removed from the supernatant by successive precipitations with $MgCl_2$ and protamine sulfate. This crude preparation was utilized as the enzyme source in this report. Details of the enzyme are given in the legend to table 1. Crude polymerase from L1210 cells was prepared by the method of Scolnick et al. [7] and stored overnight at −20° to reduce levels of a labile inhibitor.

3. Results and discussion

The data (see table 1) indicate that several of the compounds studied were inhibitory to the RNA-directed DNA polymerase. The order of greatest inhibition was adriamycin > daunomycin > prothidium Br > anthramycin > neomycin ≃ mitomycin ≃ hydroxyurea ≃ ethidium Br ≃ formamidoxime > azaserine > camptothecin > carbidium sulfate. None of the following compounds inhibited the polymerase even at 100 μ/ml: actinomycin D, antimycin, carbomycin, chloramphenicol, cycloheximide, kanamycin, oleandomycin, rutamycin, valinomycin, vancomycin. The effect of the drug was essentially the same on each of the three enzymes. Azaserine, an antineoplastic agent [12, 13] and an agent used to delay kidney graft

Table 1
Inhibition of an RNA-dependent DNA polymerase.

System	Drug concn (μg/ml)	Enzyme					
		L5178Y (cpm/protein)	I[a] %	L5178Y (cpm/mg protein)	II[b] %	L1210 (cpm/mg protein)	III[c] %
Complete	0	2500		5600		3240	
Minus template	0	0		0		580	(18)[d]
Adriamycin	1	2119	85	4718	84	–[e]	
	10	1414	57	2381	43	2000	62
	100	211	12	581	10	630	19
Anthramycin	1	2499	100	5100	91	–	
	10	2223	90	4811	86	2700	83
	100	1419	57	2801	50	1900	59
Camptothecin	1	2501	100	5504	98	–	
	10	1921	77	4816	86	–	
	100	1591	64	4219	75	2300	71
Daunomycin	1	2019	81	4089	73	–	
	10	692	28	2119	38	2080	64
	100	481	19	1409	25	680	21
Mitomycin	1	2506	100	5413	97	–	
	10	2249	90	4281	76	–	
	100	1518	61	2909	52	–	
Neomycin	1	2409	96	5223	93	–	
	10	1519	61	4298	77	–	
	100	1419	57	3971	71	–	
Ethidium Br	1	2500	100	5439	97	–	
	10	1920	77	4811	86	–	
	100	1710	68	4011	72	–	
Prothidium Br	1	2516	101	5709	102	–	
	10	1480	59	3914	70	–	
	100	1210	48	2711	48	–	
Carbidium sulfate	1	2506	100	5609	100	–	
	10	2192	88	4810	86	–	
	100	1911	77	4018	72	–	
Hydroxyurea	1	2509	100	5509	98	–	
	10	1910	76	2091	91	–	
	100	1540	62	4062	73	–	
Formamidoxine	1	2518	101	5477	98	–	
	10	1920	77	4981	89	–	
	100	1411	56	4516	81	–	
Azaleucine	1	3090	124	7100	127	–	
	10	7429	297	10,982	196	–	
	100	10,298	408	16,863	287		
Azaserine	1	2509	100	5600	100	–	
	10	2413	97	4817	86	–	
	100	1717	69	3509	63	–	

rejection [14], had no effect on the RNA-directed DNA polymerase; the reason for this is not known, especially since the other aza-amino acid studied, azaleucine, caused an acceleration of the RNA-directed DNA polymerase.

The most potent inhibitors, adriamycin and daunomycin, are very closely related structurally. Adriamycin inhibits DNA and RNA synthesis [15]; daunomycin has been demonstrated to bind to RNA and DNA [16]. At 100 μg per ml, adriamycin inhibited the L5178Y RNA (rA·dT) directed DNA polymerase 90%; daunomycin inhibited the same polymerase 75%. The action of these drugs presumably is caused by the binding of these agents to the template or to the template-product.

Ethidium Br and the related phenanthridium compounds carbidium sulfate and prothidium Br inhibit nucleic acid synthesis in HeLa cells [17], L5178Y cells [18], and bacteria [19]. Ethidium Br is known to intercalate between the bases of DNA and cause uncoiling and reverse coiling of DNA [20, 21]. The three compounds inhibited the RNA-directed DNA synthesis at 100 μg per ml between 52 and 38% in the L5178Y II system (table 1).

Anthramycin, which was moderately inhibitory to the RNA-directed DNA polymerase in all systems, binds to DNA but not RNA [22]. Camptothecin inhibits mammalian DNA synthesis [23] but does not bind to DNA [24]. Mitomycin binds to DNA strands forming crosslinks [25]. Each of these antibiotics could inhibit the RNA-directed DNA polymerase by binding to the template or to the template product. Neomycin, an aminoglycoside antibiotic, causes misreading of mRNA [26], its inhibition of the RNA-dependent DNA polymerase reported herein is not consistent with this mechanism of action. Hydroxyurea and formamidoxime are similar compounds; both inhibit the mouse leukemic cell RNA-directed DNA polymerase. Hydroxyurea is a potent inhibitor of DNA synthesis [27, 28]; whether this inhibition is at the polymerase level is not known.

Data presented herein describe some inhibitors of the RNA-dependent DNA polymerase. Potential uses of these inhibitors are as tools for specific inhibition of this polymerase as opposed to the DNA-directed DNA polymerase and for therapeutic use, if indeed this enzyme proves to be important in leukemogenesis.

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Footnotes to table 1

a The complete system contained in a final volume of 1 ml of the following: 0.50 mg protein from the 'nucleic acid free' extract ('enzyme'); 1.0 μmole each of dATP, dCTP, and dGTP; 50 μmoles tris-HCl buffer, pH 8.5; 5.0 μmoles $MgCl_2$; 10 μCi ^{3}H-methyl-TTP (7 Ci/mmole, New England Nuclear), 20 μmoles dithiothreitol; 50 μmoles NaCl; and 36 μg of rat liver RNA ('template'). Assays were incubated at 37° for 2 hr, after which 1 mg of yeast RNA was added and the assay was made 10% in trichloroacetic acid. The resultant precipitates were washed 3 times with 10% trichloroacetic acid, dissolved in 1 N NaOH at 100°, plated on a glass fiber filter and counted in a liquid scintillation counter. Rat liver RNA (template) was purchased from General Biochemicals and treated with DNase and the enzyme removed by phenol extraction. DNase and RNase were purchased from Worthington. All data are given with endogenous activity (about 10%) determined with water substituted for the 'template' subtracted. The complete system also contained 50 μl of distilled H_2O, for drug assays the drug was present in this 50 μl.

b The complete system was exactly as in (a) except 0.01 A_{260}rA·dT was substituted for the rat liver RNA as template.

c Leukemia L1210 cells were isolated [9] from CDF_1 mice, and the crude polymerase prepared as described by Scolnick et al. [7]. The cell extract was stored overnight at −20° to reduce levels of a labile inhibitor of the reaction. The complete system, in a final volume of 100 μl, contained: 20 mM TES buffer, pH 7.4; 60 mM KCl, 1 mM Mn acetate; 2 mM dithiothreitol; 0.01 A_{260}rA·rU; 10 μg enzyme protein and 10 μCi ^{3}H-TTP. Incubations were terminated after 60 min at 37° by addition of 5% trichloroacetic acid. The precipitates were washed with 5% trichloroacetic acid (4°), 70% ethanol and 99% ethanol, on Millipore filters (HA), then radioactivity was measured by liquid scintillation counting.

d Percent the drug assay is of the complete.

e Experiment not performed.

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