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Preferential Inhibition of Terminal Deoxynucleotidyltransferase Activity among Deoxyribonucleic Acid Polymerase Activities of Leukemic and Normal Cells by Geldanamycin, Streptoval C, Streptovarone, and Dapmavarone

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SUMMARY

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Geldanamycin, streptoval C, streptovarone, and dapmavarone preferentially inhibited terminal deoxynucleotidyltransferase activity of Molt-4 cells and leukocytes from an acute lymphoblastic leukemia patient as compared with DNA polymerase α, β, and γ activities of these cells or of phytohemagglutinin-stimulated normal human lymphocytes. Streptovaricin C, the parent compound from which streptoval C, streptovarone, and dapmavarone are derived, was a poor inhibitor of these enzyme activities. Geldanamycin, streptoval C, and streptovarone inhibited terminal deoxynucleotidyltransferase activity more than reverse transcriptase activity of simian sarcoma virus, but dapmavarone inhibited these enzyme activities about the same. Inhibition of terminal deoxynucleotidyltransferase activity by these compounds was reversed by dilution but not by addition of extra initiator [$(dA)_{12-18}$], a divalent cation (Mn^{2+}), bovine serum albumin, or substrate (dGTP). Prior incubation of each compound with the transferase resulted in greater inhibition than prior incubation with initiator or lack of prior incubation. These findings suggest that geldanamycin, streptoval C, streptovarone, and dapmavarone preferentially inhibit terminal deoxynucleotidyltransferase activity by reversibly binding to the enzyme and not to initiator or divalent cation.

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INTRODUCTION

Terminal deoxynucleotidyltransferase (EC 2.7.7.31) catalyzes the polymerization of deoxyribonucleotides on the 3'-hydroxyl ends of oligo- or polydeoxyribonucleotide initiators (1). Originally considered specific for thymus, this enzyme is found in large amounts in leukocytes of acute lymphoblastic leukemia (2) and rare cases of both acute (3) and chronic (4, 5) myelocytic leukemias, but is barely detectable in normal leukocytes or leukocytes from chronic lymphocytic leukemia and in most cases of chronic and acute myelocytic leukemias. In acute lymphoblastic leukemia, the specific activity of terminal deoxynucleotidyltransferase changes with the status of disease, i.e., high upon diagnosis, low upon remission, and high upon relapse (2, 6). Thus this enzyme may have an important function in certain leukemic cells, and selective inhibitors of its activity may have chemotherapeutic potential. In our search for potential inhibitors of terminal deoxynucleotidyltransferase, we have found that geldanamycin and the streptovaricin degradation products streptoval C, streptovarone, and dapmavarone (Fig. 1) (7) preferentially inhibit terminal deoxynucleotidyltransferase activity as compared with DNA polymerase α, β, and γ (EC 2.7.7.7) (8) activities of either neoplastic or normal cells. Geldanamycin, like streptovaricin, is an ansamycin antibiotic; i.e., it has an aromatic ring system spanned by an aliphatic bridge (7).

MATERIALS AND METHODS

Cells and enzyme preparation. Molt-4 cells are of acute lymphoblastic leukemic origin (9). Their culture and harvesting have been described (9). Both leukocytes from an acute lymphoblastic leukemia patient and phytohemagglutinin-stimulated normal human lymphocytes were obtained as described previously (9). Terminal deoxynucleotidyltransferase and DNA polymerases α, β, and γ were prepared according to our published procedures (9, 10). DNA polymerases α, β, and γ were prepared from all the above cells, whereas terminal deoxynucleotidyltransferase was prepared from Molt-4 and acute lymphoblastic leukemia

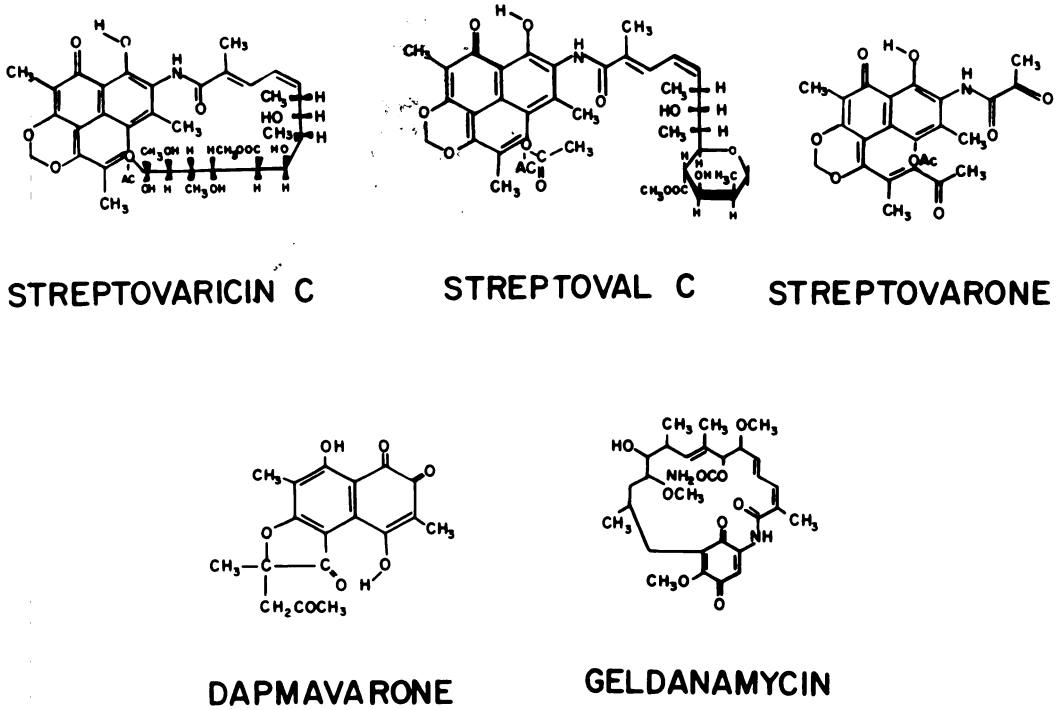

FIG. 1. *Structures of the compounds studied*
See Rinehart and Shield (7) for more information regarding these compounds.

cells, since phytohemagglutinin-stimulated normal human lymphocytes have little terminal deoxynucleotidyltransferase activity (9). The specific activities of these enzymes were similar to those reported previously (10). Simian sarcoma virus reverse transcriptase was prepared according to Abrell and Gallo (11), and its properties and specific activity were comparable to their reported results (11).

Enzyme assays. Enzymes were assayed as follows (in a 0.2-ml final volume). For DNA polymerase α or β, the reaction mixture contained 10 μmoles of Tris-HCl (pH 8.3), 1.2 μmoles of magnesium acetate, 4.0 μmoles of dithiothreitol, 0.16 μmole each of dATP, dGTP, and dCTP, 0.01 μmole of [^{3}H]dTTP (144 cpm/pmole), 30 μg of activated (9) calf thymus DNA (Worthington), and either DNA polymerase α (0.3 μg of protein) or β(0.8 μg of protein).

For DNA polymerase γ, the reaction mixture contained 10 μmoles of Tris-HCl (pH 7.5), 0.1 μmole of $MnCl_2$, 20 μmoles of KCl, 0.6 μmole of dithiothreitol, 8 μg of bovine serum albumin, 0.001 μmole of [^{3}H]dTTP (1036 cpm/pmole), 2.4 μg of poly(A)·$(dT)_{12\text{-}18}$ (Schwarz/Mann), and 0.12 μg of enzyme protein.

The assay mixture for terminal deoxynucleotidyltransferase contained 10 μmoles of Tris-HCl (pH 7.5), 0.1 μmole of $MnCl_2$, 20 μmoles of KCl, 0.6 μmole of dithiothreitol, 8 μg of bovine serum albumin, 0.01 μmole of [^{3}H]dGTP (115 cpm/pmole), 2.4 μg of $(dA)_{12\text{-}18}$ (Collaborative Research, Inc.), and 0.4 μg of enzyme protein.

The reaction mixture for simian sarcoma virus reverse transcriptase was the same as for DNA polymerase γ, except that 0.0003 μmole of [^{3}H]dTTP (8910 cpm/pmole) and 0.06 μg of enzyme protein were used.

Unless mentioned otherwise, the addition sequence involved template/primer or initiator, either geldanamycin, streptovaricin C, streptoval C, streptovarone, or dapmavarone, the other components of the reaction mixture, and finally enzyme. After 30 min of incubation at 37° (30° for DNA polymerase γ), 50 μg of yeast RNA and 1 ml of 20% trichloracetic acid containing 3% sodium pyrophosphate were added. The precipitates were collected on nitrocellulose membrane filters, washed with 5% trichloracetic acid, dried, and counted using a toluene-based scintillation fluid (9). All enzyme activities were linear with respect to time and protein concentration.

Geldanamycin and streptovaricin C were supplied by the Upjohn Company, and streptovarone, streptoval C, and dapmavarone were prepared as described (12, 13).

RESULTS AND DISCUSSION

Streptoval C, streptovarone, dapmavarone, and geldanamycin inhibited terminal deoxynucleotidyltransferase activity of leukocytes from an acute lymphoblastic leukemia patient (Fig. 2). These compounds were less potent inhibitors of DNA polymerase α, β, and γ activities of these cells. DNA polymerase γ activity was more sensitive than DNA polymerase α and β activities to inhibition by streptoval C, streptovarone, and geldanamycin, whereas DNA polymerase α, β, and γ activities were inhibited to a similar degree by dapmavarone (Fig. 2). Qualitatively similar results were obtained with terminal deoxynucleotidyltransferase of Molt-4 cells and DNA polymerases α, β, and γ of Molt-4 cells and phytohemagglutinin-stimulated normal human lymphocytes (data not shown). Streptovaricin C, the parent compound from which streptoval C, streptovarone, and dapmavarone are derived by cleavage and degradation (Fig. 1) (7), inhibited the activities of terminal deoxynucleotidyltransferase and DNA polymerases α, β, and γ by less than 25% at 0.52 mM (Fig. 3). Thus the preferential and increased inhibition of terminal deoxynucleotidyltransferase by streptoval C, streptovarone, and dapmavarone may be related to cleavage of the ansamycin structure and/or a reduction in molecular weight. Geldanamycin also is lower in molecular weight than streptovaricin C, but has an intact ansamycin structure.

Streptoval C and streptovarone inhibited terminal deoxynucleotidyltransferase activity more than simian sarcoma virus reverse transcriptase activity, but dapmavarone inhibited these two enzyme activities to about the same degree (Fig. 2). Streptoval C, streptovarone, and dapmavarone were previously reported to inhibit the endogenous

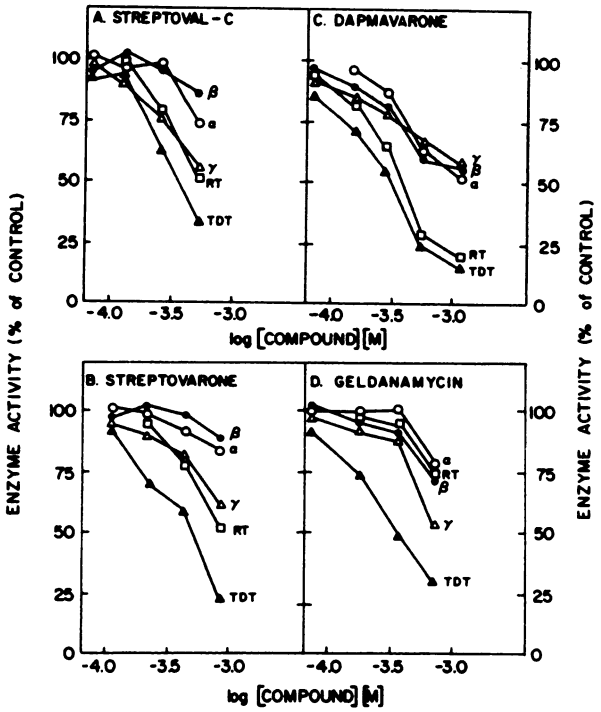

FIG. 2. *Effects of streptoval C, streptovarone, dapmavarone, and geldanamycin on activities of terminal deoxynucleotidyltransferase (TDT) and DNA polymerases α, β, and γ from leukocytes of an acute lymphoblastic leukemic patient and reverse transcriptase (RT) from simian sarcoma virus*

Controls without each compound incorporated 60.5, 72.3, 58.7, 9.4, and 0.6 pmoles of tritium-labeled nucleoside monophosphate for terminal deoxynucleotidyltransferase, DNA polymerases α, β, and γ, and reverse transcriptase, respectively.

reverse transcriptase activity of Rauscher leukemia virus (14). The concentrations of these compounds used to inhibit the endogenous activity were lower than those found to inhibit the activity of the purified simian sarcoma virus enzyme assayed with poly(A)·$(dT)_{12-18}$. This may be due to differences in enzyme source and/or assay conditions. On an equimolar basis, geldanamycin inhibited simian sarcoma virus reverse transcriptase activity to a lesser degree than the three streptovaricin derivatives (Fig. 2), which correlates closely with the results obtained with Rauscher leukemia virus reverse transcriptase (14, 15). Nonetheless, geldanamycin also inhibited terminal deoxynucleotidyltransferase activity much more than reverse transcriptase activity (Fig. 2).

The inhibition of terminal deoxynucleotidyltransferase by either streptoval C, streptovarone, dapmavarone, or geldanamycin was reversed by dilution (Table 1), Prior incubation of either streptoval C, streptovarone, dapmavarone, or geldanamycin with terminal deoxynucleotidyltransferase yielded greater inhibition than prior incubation of these compounds with initiator [$(dA)_{12-18}$] or lack of prior incubation (Table 2). Addition to standard reaction mixtures of either extra bovine serum albumin, initiator, Mn^{2+}, or dGTP had no effect on inhibition of terminal deoxynucleotidyltransferase by either streptoval C, streptovarone, dapmavarone, or geldanamycin (Table 3). All these results indicate that streptoval C, streptovarone, dapmavarone, and geldanamycin inhibit terminal deoxynucleotidyltransferase activity by reversibly binding to enzyme and not to initiator or divalent cation and that inhibition is relatively specific, as high concentrations of a foreign protein (bovine serum albumin) did not reverse inhibition. Also, these inhibitors probably do not interfere with substrate (dGTP) utilization, because a 10-fold

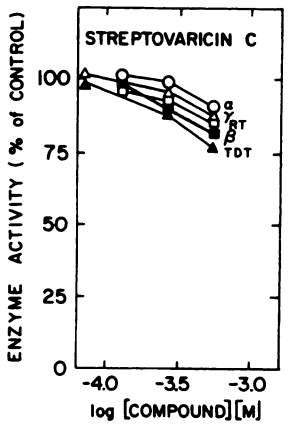

FIG. 3. *Effect of streptovaricin C on activities of terminal deoxynucleotidyltransferase (TDT) and DNA polymerases α, β, and γ from leukocytes of an acute lymphoblastic leukemia patient and reverse transcriptase (RT) from simian sarcoma virus*

Controls without streptovaricin C incorporated 63.1, 69.5, 57.3, 10.2, and 0.6 pmoles of tritium-labeled nucleoside monophosphate for terminal deoxynucleotidyltransferase, DNA polymerases α, β, and γ, and reverse transcriptase, respectively.

TABLE 1

Effect of dilution on inhibition of terminal deoxyribonucleotidyltransferase by geldanamycin, streptoval C, streptovarone, or dapmavarone

Duplicate standard assay mixtures (control) and duplicate experimental mixtures containing the indicated compounds were incubated at 37°. After 1 min, one of the experimental and one of the control mixtures were diluted 10 times into complete assay mixtures lacking enzyme and inhibitor but containing bovine serum albumin (200 μg/ml). After 30 min at 37°, the assay mixtures were processed as described in MATERIALS AND METHODS.

Inhibitor	dGMP incorporated	
	Undiluted	Diluted
	pmoles	
None (control)	60	56
0.37 mM geldanamycin	33	55
0.52 mM streptoval C	22	51
0.43 mM streptovarone	38	53
0.58 mM dapmavarone	20	58

excess of dGTP failed to overcome inhibition (Table 3).

In conclusion, streptoval C, streptovarone, dapmavarone, and geldanamycin are preferential inhibitors of terminal deoxynucleotidyltransferase activity as compared with the activities of DNA polymerases α, β, and γ of both neoplastic and normal cells. These compounds are also more potent inhibitors of terminal deoxynucleotidyltransferase than streptovaricin C, the parent compound of streptoval C, streptovarone,

TABLE 2

Prior incubation of geldanamycin, streptoval C, streptovarone, or dapmavarone with terminal deoxyribonucleotidyltransferase or $(dA)_{12-18}$ and subsequent inhibition of the enzyme

Each inhibitor (geldanamycin, streptoval C, streptovarone, or dapmavarone) was incubated for 5 min at 37° with either terminal deoxynucleotidyltransferase or $(dA)_{12-18}$, followed by addition of the remaining constituents of the reaction mixtures and standard assay. Assays were also conducted in which none of the components of the reaction mixtures were first incubated with an inhibitor.

Initial incubation components	dGMP incorporated		Enzyme activity
	− Inhibitor (controls)	+ Inhibitor	
	pmoles		*% control*
Enzyme (±) geldanamycin (0.37 mM)	59	20	34
$(dA)_{12-18}$ (±) geldanamycin (0.37 mM)	65	33	51
None	62	35	57
Enzyme (±) streptoval C (0.52 mM)	55	6	11
$(dA)_{12-18}$ (±) streptoval C (0.52 mM)	60	25	42
None	58	22	38
Enzyme (±) streptovarone (0.43 mM)	60	24	40
$(dA)_{12-18}$ (±) streptovarone (0.43 mM)	63	39	62
None	64	38	59
Enzyme (±) dapmavarone (0.58 mM)	54	5	9
$(dA)_{12-18}$ (±) dapmavarone (0.58 mM)	61	21	34
None	55	17	31

TABLE 3

Effects of various additions to standard assay mixtures on inhibition of terminal deoxynucleotidyltransferase by geldanamycin, streptoval C, streptovarone, or dapmavarone

Standard assay mixtures (0.2 ml, final volume) for terminal deoxynucleotidyltransferase contained 2.4 μg of $(dA)_{12-18}$, 8 μg of bovine serum albumin, 0.1 μmole of Mn^{2+}, and 0.01 μmole of dGTP. Other components of the standard assay mixtures are listed in MATERIALS AND METHODS.

Additions to standard assay mixtures	Enzyme activity				
	Inhibitor (controls)	Geldanamycin (0.37 mM)	Streptoval C (0.26 mM)	Streptovarone (0.43 mM)	Dapmavarone (0.29 mM)
	% control				
None	100 (63)[a]	50	65	59	54
51.6 μg of $(dA)_{12-18}$	100 (74)	54	68	63	52
50 μg of bovine serum albumin	100 (69)	51	66	60	57
1.0 μmole of Mn^{2+}	100 (49)	48	62	56	51
0.1 μmole of dGTP	100 (61)	55	61	64	49

[a] Numbers in parentheses are the picomoles of dGMP incorporated.

and dapmavarone. Since terminal deoxynucleotidyltransferase is found in large amounts in certain leukemic cells and may have an important function in these cells, these preferential inhibitors of terminal deoxynucleotidyltransferase activity may have chemotherapeutic value. Indeed, streptoval C was 5–10 times more cytotoxic to the lymphoid leukemic cell line, Molt-4, than streptovaricin C.[1] Furthermore, streptoval C, streptovarone, and dapmavarone have additional chemotherapeutic interest because they inhibited reverse transcriptase of RNA tumor viruses more potently than their parent compound, streptovaricin C (Figs. 2 and 3) (14), and because streptoval C and streptovarone were more active against Rauscher leukemia virus than streptovaricin C (16).

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[1] Unpublished observations.