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Selective Killing of Leukemia Cells by Inhibition of TdT *

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A. Introduction

Terminal deoxynucleotidyl transferase (TdT) is a unique DNA synthetic enzyme whose expression in normal cells is restricted to subsets of primitive lymphocytes, and among neoplastic cells is found only in the blast cells of some forms of acute leukemia and diffuse lymphoma [1–5]. This polymerase has achieved increasing attention over the last several years from both basic biologists and physicians caring for patients with malignant hematologic disease. For biologists it has emerged as a useful biochemical marker for subsets of pre-B and pre-T cells, and has played an important role in studies aimed at dissecting the ontogeny of the lymphoid system [6, 7]. In clinical medicine, blast cell TdT assays have been shown to be useful in the subclassification of acute leukemias and diffuse lymphomas, and TdT status has been used to assign patients to therapeutically meaningful categories [8, 9].

Although the existence of this enzyme has been known for over 20 years, the function it subserves in the cells in which it is found is presently unknown. Its strict limitation in normal animals to lymphoid cells during the early phases of their differentiation suggests that it may play a critical role in this process. Presumably TdT subserves a similar role in leukemia cells, although the process of differentiation is itself disturbed. Whatever its role might be, we asked whether inhibition of TdT in leukemic cells might constitute a lethal event to such cells. We therefore began a study to find a specific TdT inhibitor. With such a probe one would be able to examine a variety of cellular processes and functions in TdT-positive cells, and gain insight into the role of this enzyme in both normal and malignant cells.

B. In Vitro Inhibition of TdT Catalysis

We began our search for TdT inhibitors by screening a series of 6-anilinouracil derivates for their ability to inhibit TdT catalysis as measured in a standard biochemical reaction. This series of compounds was selected for our initial screen because of the work of Wright, Baril, and Brown [10] with one such compound, 6-butylanilinouracil, which they showed to be a specific inhibitor of HeLa cell DNA polymerase α. The other constitutive HeLa cell polymerases, polymerases β and γ, were not inhibited. Furthermore, this compound, in a range of concentrations which were inhibitory to in vitro enzyme catalysis, also strongly inhibited cell proliferation in nonsynchronous HeLa cell cultures in a dose-dependent fashion. Similar data on growth inhibition were also generated with KB cells lines. We thus proceeded to test related analogues for their effect on TdT.

Fifteen uracil analogues were studied. Each was employed at 200 μmolar final concentration in 1% DMSO (required for solubility) in our standard TdT biochemical assay [1]. Control TdT reactions were also run in the presence of 1% DMSO. Two

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Designator	Name	nmoles ^{3}H-dGMP inc.
Control		1.22
GW-9E	6-anilinouracil	1.21
GW-7B	6-(benzylamino)uracil	1.03
GW-7C	6-(phenetylamino)uracil	1.31
GW-11D	6-(p-butylanilino)uracil	1.12
GW-22E	6-(p-hydroxyanilino)uracil	1.21
GW-16C	6-(p-acetamidobenzylamino)uracil	1.43
GW-18B	6-(cyclohexylamino)uracil	1.31
GW-20B	6-(cyclohexylmethylamino)uracil	1.33
GW-18E	6-(*n*-pentylamino)uracil	1.20
GW-22A	6-(iso-pentylamino)uracil	1.21
GW-17B	6-(3′,4′-trimethyleneanilino)uracil	1.23
GW-28A	6-(*d*-naphthylamino)uracil	1.24
GW-33E	5-(p-methoxybenzyl)-6-aminouracil	1.20
GW-17E	6-(p-methoxyanilino)uracil	0.51
GW-18C	6-(p-aminoanilino)uracil	0.69

Table 1. Effect of uracil analogues on TdT activity

compounds, designated GW-17E and GW-18C, showed significant inhibition in this screen (Table 1).

This inhibition by GW-17E and GW-18C was specific for TdT. Data for representative experiments involving polymerases α, β, and γ are shown in Table 2. Both compounds inhibited TdT in a dose-dependent manner (Fig. 1). Avian myeloblastosis virus reverse transcriptase was not inhibited by either GW-17E or GW-18C.

The general structure of compounds GW-17E and GW-18C are shown Fig. 2. For compound 17E the R denotes a methoxy substitution; for 18C the R denotes an amino substitution. Para position substitutions are critical for inhibitory activity. Moving the methoxy or amino group to another position on the aniline ring results in loss of inhibitory activity.

In these preliminary studies inhibition was neither initiator dependent nor substrate dependent: it occurred with both oligo(dA) and single-stranded DNA as initiators and with dGTP and TTP as substrates. The TdT used in these experiments was purified (using ion-exchange and affinity chromatography [12]) from both calf

Table 2. Specificity of inhibition of TdT by GW-17E and GW-18C

Enzyme	CPM ^{3}H-dNMP incorporated		
	Control	+17E	+18C
TdT	12,130	4,900	3,100
Pol α	15,040	15,217	15,417
Pol β	5,221	5,492	5,218
Pol γ	7,490	7,223	7,165

Effect of 400 μmolar inhibitor on activity of homogeneously purified human leukemic TdT and HeLa cell DNA polymerases. TdT, pol α, pol β, and pol γ were assayed as previously described [10, 11]

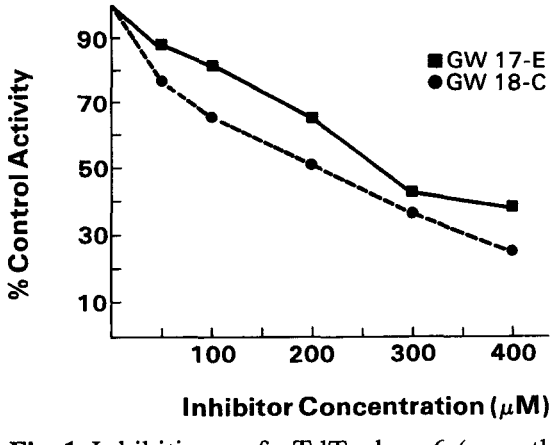

Fig. 1. Inhibition of TdT by 6-(p-methoxyanilino)uracil (GW-17E) and 6-(p-aminoanilino)uracil (GW-18C)

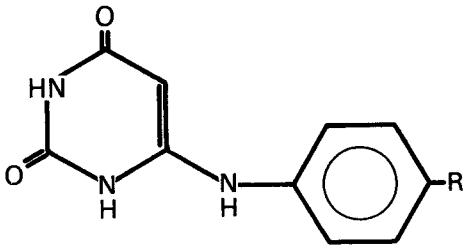

Fig. 2. General structure of compounds GW-17E and GW-18C. For 17E the R denotes a methoxy substitution, and for 18C the R denotes an amino substitution

thymus gland and human leukemia cells. Formal inhibition studies to determine the nature of the inhibition (competitive versus noncompetitive K_i values) are in progress.

C. Effect of GW-17E and GW-18C on Cell Proliferation

We next attempted to extend these observations to additional TdT-positive and TdT-negative cell lines. We studied 30 cell lines initiated from patients with various forms of acute leukemia and maintained at Roswell Park Memorial Institute. Twenty-one lines were TdT positive, nine were TdT negative. However, the requirement for 1% DMSO for the solubility of GW-17E and GW-18C turned out to be toxic for these cell lines: control cultures grew with extreme variability in the presence of 1% DMSO, making the interpretation of inhibitor effects impossible.

Table 3. Effect of GW-17E and GW-18C on cell proliferation

Cell line	Source	TdT status	Growth
HeLa	Human	Negative	Unchanged
L1210	Murine	Negative	Unchanged
LE-4	Murine	Positive	Markedly inhibited
HPB-ALL[a]	Human	Positive	Control cultures fail to grow in 1% DMSO
JM[a]	Human	Positive	

[a] The data noted for the two human leukemia cell lines are representative of all 30 lines studied

Work in progress now is directed at the development of compounds related to GW-17E and GW-18C, which are both inhibitory and water soluble. One such compound, designated 20-DN (Fig. 3), has been identified. At 100 μmolar concentration this compound inhibits TdT in vitro by 85% of control, while polymerases α, β, and γ are not inhibited, even at 500 μmolar concentration.

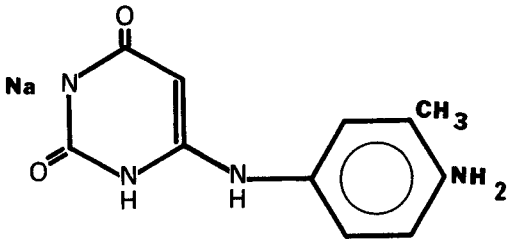

Fig. 3. Structure of compound GW-20DN

It should be possible to determine, using a panel of human and animal TdT-positive and TdT-negative cell lines, whether the results we have seen with HeLa, L1210, and EL-4 cells are general and related to TdT states, and not restricted to the lines studied to date.

D. Conclusion

The physiologic function of TdT in cells in which it is expressed, either leukemic or normal, is presently unknown. Its strict limitation in normal animals to lymphoid cells during the early phases of their differentiation suggests that it may play a critical role in this process. Presumably TdT subserves a similar role in leukemia cells, although the process of differentiation in these cells is itself disturbed. Whatever its role might be, we have asked whether inhibition of TdT in leukemic cells might constitute a lethal event to such cells. Our preliminary data on growth inhibition by GW-17E and GW-18C of a murine TdT-positive cell line suggests that this may indeed be the case.

The development of potent and specific TdT inhibitors will provide a critical tool in the dissection of the biological role of TdT. Such compounds may be of therapeutic use

in TdT-positive malignant states as definitive or adjunctive therapy, or possibly in the in vitro destruction of TdT-positive malignant cells in bone marrow prior to autologous grafting. Although TdT-positive normal cells, in addition to TdT-positive malignant cells, might be eliminated by therapy of this short, the fact that pluripotent stem cells are TdT negative suggests that the normal TdT-positive cell compartment would therefore be renewable.

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