Cytidine Triphosphate Synthetase Activity in Lymphoproliferative Disorders

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Abstract

Cytidine triphosphate synthetase (CTP synthetase) activity was measured in extracts from normal and malignant lymphoid cells. A range of enzyme activities was found in the majority of the lymphoproliferative disorders examined, with acute lymphocytic leukemia, nodular poorly differentiated lymphocytic lymphoma, and diffuse histiocytic (large cell) lymphoma (DHL) types exhibiting the widest ranges. The highest levels occurred in acute lymphocytic leukemia, nodular poorly differentiated lymphocytic lymphoma transforming to DHL, and DHL. In chronic lymphocytic leukemia and diffuse well-differentiated lymphocytic lymphoma, a narrow range was encountered, with individual values falling within that of the controls. The highest CTP synthetase levels in Hodgkin’s disease were found in the clinically aggressive lymphocyte-depleted Hodgkin’s disease. Statistically significant differences were found between the distributions of CTP synthetase activities in acute lymphocytic leukemia and the chronic leukemias (p < 0.01) and between favorable histological types of non-Hodgkin’s lymphoma (diffuse poorly differentiated lymphocytic lymphoma) and the unfavorable DHL category (p < 0.05). It is suggested that CTP synthetase activity is a biochemical marker of the clinical aggressiveness of malignant lymphoma. There was not a close correlation (r² = 0.52) between CTP synthetase and thymidine kinase activities, indicating that other biological factors in addition to cell proliferative rate influence the intracellular CTP synthetase level. Furthermore, in view of the heterogeneity of CTP synthetase activity in leukemia and lymphoma, it is likely that the preexisting enzyme level will be an important determinant of the efficacy of the investigational antileukemic agent 3-deazaaduridine, which is thought to act by inhibiting CTP synthetase.

Introduction

CTP synthetase (UTP:L-glutamine ligase, EC 6.3.4.2) catalyzes the formation of CTP from the substrate UTP. The precise biochemical role of mammalian CTP synthetase activity is uncertain, but the enzyme is thought to play an important part in the determination of cellular CTP and dCTP pools. Recent data suggest that, in addition to this physiological role, CTP synthetase is uniquely important in the pyrimidine metabolism of mammalian tumors. It has been established that there is a specific increase in the CTP synthetase activity and content of rat hepatomas, which is thought to be a marker of the proliferative rate and biological aggressiveness of these tumors. Preliminary data show that there is an increase in the CTP synthetase activity of other rodent tumors and in human renal cell carcinoma, but the tumor specificity of this phenomenon is unclear.

Materials and Methods

Materials. [5,6-³H]UTP (43 Ci/mmol) and [6-³H]thymidine (5 Ci/mmol) were purchased from the Radiochemical Centre, Amersham, England. Nucleotides and Dowex 50WX8 (200 to 400 mesh) were obtained from Sigma Chemical Co., St. Louis, Mo. Polygram CEL 300 PEI/UV254 thin-layer chromatography plates were supplied by Brinkmann, Inc., New York, N. Y. PCS scintillation counting fluid was obtained from Amersham, Amersham, England. All other chemicals used were of analytical grade and were obtained from commercial sources.

Patients Studied. Peripheral blood lymphocytes were obtained from a control group, which contained 10 normal volunteers and 9 patients with nonneoplastic disorders. Seven lymph nodes obtained at surgical biopsy and showing normal histology or minimal reactive changes formed a second control group.

Patients with lymphoproliferative disorders were all previously untreated. They included 11 patients with B-CLL, all of whom exhibited an absolute peripheral blood lymphocytosis (range 15,600 to 68,000/...
with the majority of cells exhibiting faint immunofluorescence for monomolecular IgM or IgM-IgD and mouse erythrocyte receptor positivity. Two additional patients with CLL were identified as T-CLL by the ability of the majority of peripheral blood lymphocytes (range 30,960 to 75,800/µl) to spontaneously bind sheep erythrocytes. Anti-T monomolecular antibody studies showed that the majority of peripheral blood lymphocytes expressed the phenotype of helper T-lymphocytes (OKT4) in one patient with T-CLL, whereas, in the second patient, the peripheral blood lymphocytes exhibited the phenotypic features of suppressor T-lymphocytes (OKT8). One patient had HCL with more than 80% of the peripheral blood lymphocytes (32,350/µl) being abnormal and exhibiting tartrate-resistant acid phosphatase activity. Seven patients had ALL. Lymphoblasts were the predominant peripheral blood mononuclear cell in all 7 patients. Peripheral blood lymphoblasts from 2 patients (range 8,430 to 21,100/µl) were identified as Thy-ALL by their ability to form spontaneous rosettes with sheep erythrocytes. Peripheral lymphoblasts from 4 patients (range 4,380 to 12,100/µl) did not show T- or B-lymphocyte surface markers but did express the C-ALL antigen and were classified as C-ALL. One patient was considered to have null-ALL on the basis that the peripheral blood lymphoblasts (5,760/µl) did not express T- or B-cell markers or the C-ALL antigen.

Lymph nodes were obtained at surgical biopsy, and the histological and immunological features were independently reviewed by G. Medley. In 41 patients, the histological findings were those of NHL, which were categorized according to the modified Rappaport classification (2). Individual categories and numbers were: DWDLL, 5; NPDLL, 14; PDPLL, 6; and DHL, 13. Lymph node biopsies from 8 patients exhibited histological features of HD with subtypes (15) and numerical distribution being nodular sclerosing HD, 4; mixed-cellularity HD, 1; and lymphocyte-depleted HD, 3.

The Wilcoxon rank sum test was used to compare the CTP synthetase activity among the various types of lymphoproliferative disorders. Correlation between CTP synthetase and thymidine kinase activities was assessed by construction of a scatter diagram and linear regression analysis of the data.

**Sample Preparation.** Normal and leukemic peripheral blood lymphocytes were obtained from 10 ml of heparinized venous blood by centrifugation on Ficoll-Hypaque (4). The lymphocytes were suspended at 10⁷ cells/ml of 50 mM Tris-HCl buffer, pH 8.5, containing 5 mM dithiothreitol and 10 mM L-glutamine (Buffer A) and lyzed by rapid freeze-thawing twice in liquid nitrogen. The cell extract was then centrifuged at 10,000 x g at 4° for 15 min, and the supernatant was assayed for CTP synthetase activity. Samples used to measure thymidine kinase activity were prepared as described previously (9).

Lymph nodes obtained at biopsy were divided into sections for histological examination, special investigations including immunological tests, and CTP synthetase determination. After being rinsed twice with cold 0.9% NaCl solution, samples (0.1 to 0.5 g) were suspended in 2 volumes (w/v) of Buffer A and subjected to Dounce homogenization. The homogenate was centrifuged at 10,000 x g at 4° for 15 min, and the supernatant was assayed for CTP synthetase activity. Samples used to measure thymidine kinase activity were prepared as described previously (9).

**CTP Synthetase Assay.** The enzyme assay contained, in a total volume of 0.1 ml, 50 mM Tris-HCl (pH 8.5), 0.25 mM UTP (5 µCi/µmol), 5 mM ATP, 0.5 mM GTP, 10 mM MgCl₂, 10 mM L-glutamine, 10 mM dithiothreitol, and the enzyme preparation (50 µl). A pH of 8.5 was chosen as the human enzyme has a broad pH optimum between pH 8.4 and 9.4. Reactions were carried out at 37° for 45 min. The reaction was terminated by adding 10 µl of 60% perchloric acid to the reaction on ice. Precipitated protein was removed by centrifugation at 10,000 x g at 4° for 10 min. The CTP formed was determined by acid hydrolysis of [³H]CTP and [³H]UTP to the nucleoside monophosphates (21). [³H]CMP was then separated by chromatography on Dowex 50-H⁺ ion-exchange resin (21). The identity of the reaction product and the validity of this procedure was confirmed by polyethyleneimine cellulose thin-layer chromatography (26). Under the experimental conditions used, 82 to 87% of [³H]CTP was recovered as [³H]CMP. The reaction was linear with respect to protein concentration and up to 75 min. Enzyme activity was expressed as nmol of CTP formed per hr per mg of protein.

**Thymidine Kinase Assay.** Thymidine kinase was assayed as previously described using [6-³H]thymidine as the radiolabeled substrate (8, 9).

Protein was determined by the method of Bradford (5) using bovine serum albumin as the standard.

**RESULTS**

**CTP Synthetase Activity in Control Blood Lymphocytes and Lymph Nodes.** Peripheral blood lymphocytes from normal individuals and patients with nonneoplastic medical disorders had a mean CTP synthetase activity of 0.91 ± 0.20 (S.D.) nmol/hr/mg of protein (range, 0.78 to 1.30). Tissue extracts from normal lymph nodes and those exhibiting minimal reactive changes had a mean enzyme activity of 1.18 ± 0.19 nmol/hr/mg of protein (range, 0.95 to 1.40).

**CTP Synthetase Activity in ALL and Chronic Leukemias.** The distribution of CTP synthetase activity in the ALL and chronic leukemias is shown in Chart 1. There was a statistically significant difference (p < 0.01) in the distribution of enzyme activities between ALL and the chronic leukemias.

CTP synthetase activity in ALL was 3.33 nmol/hr/mg of protein, 3.70-fold the mean control level. There was considerable variation in individual levels, with CTP synthetase activities ranging from 1.30 to 6.70 nmol/hr/mg of protein. The highest values were found in 3 examples of C-ALL, but elevated CTP synthetase activity was also found in lymphoblast extracts from 2 examples of Thy-ALL. One of the patients with C-ALL and both with Thy-ALL had disease resistant to current antileukemic agents. The 2 other C-ALL patients with high CTP synthetase levels achieved complete remission following therapy with a vincristine-prednisolone-Adriamycin regimen of treatment (28), as did the one patient with null-ALL and one patient with C-ALL who showed CTP synthetase activity within or close to the control range.

The mean CTP synthetase activity in B-ALL of 0.85 nmol/hr/mg of protein closely approximated that of the control...
group, and the range of individual enzyme levels fell within or near that of the controls. Similarly, the CTP synthetase activity of a single example of HCL (1.10 nmol/hr/mg of protein) fell within the control range. Two examples of T-CLL had CTP synthetase activities greater than the upper control range, with the individual values being 1.60 and 1.75 nmol/hr/mg of protein, respectively.

**CTP Synthetase Activity in NHL and HD.** The distribution of CTP synthetase activities in NHL grouped according to the modified Rappaport scheme (2) and in the subtypes of HD (15) is shown in Chart 2.

Statistically significant differences were not observed in the distribution of CTP synthetase activities in the various categories of NHL. However, when enzyme values for tumors exhibiting histological progression to DHL were excluded from the analysis, a statistically significant difference ($p < 0.05$) was found between the distribution of enzyme activities in each of the favorable categories (DWDLL, NPDLL, and DPDDL) and that in the unfavorable DHL category. In DWDLL, the mean enzyme activity (1.06 nmol/hr/mg of protein) and range of activities fell within that of the controls. There was considerable heterogeneity in the CTP synthetase activities of the other NHL categories examined, with individual enzyme levels ranging from values below the control range to almost 4-fold the mean control activity. The NPDLL category exhibited the widest range of activities, with values ranging from 0.42 to 4.10 nmol/hr/mg of protein. The mean enzyme activity of 1.34 nmol/hr/mg of protein was above the control range. Two of the NPDLL patients with high CTP synthetase activity showed histological evidence of transformation to the unfavorable DHL category. The mean enzyme activity and range for DPDDL were 1.67 (range, 0.80 to 3.30) nmol/hr/mg of protein. In this category, the highest CTP synthetase activity occurred in lymph node extracts from a patient who had previously been documented to have NPDLL and for whom there was morphological evidence of further progression to DHL. The DHL category exhibited the highest mean enzyme activity (2.25 nmol/hr/mg of protein), together with a range of individual levels, with values occurring below the control range to greater than 3-fold the mean control activity. The mean enzyme activity in HD was 1.26 (range, 0.45 to 3.00) nmol/hr/mg of protein. The range of CTP synthetase activities in HD correlated with the histological subtypes of HD. The individual enzyme activities in nodular sclerosing HD and mixed-cellularity HD fell within or below the control range, while the CTP synthetase activity [2.25 (range, 1.75 to 3.00) nmol/hr/mg of protein] was above the control range in all 3 examples of the clinically aggressive lymphocyte-depleted HD.

The mean values and range of CTP synthetase activities in the controls and the various types of lymphoproliferative disorders are summarized in Table 1.

**Table 1**

<table>
<thead>
<tr>
<th>Peripheral blood lymphocytes</th>
<th>CTP synthetase activity (nmol/hr/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (19)$^a$</td>
<td>0.91$^b$ (0.78–1.30)$^c$</td>
</tr>
<tr>
<td>B-CLL (11)</td>
<td>0.85 (0.50–1.60)</td>
</tr>
<tr>
<td>T-CLL (2)</td>
<td>1.68 (1.60–1.75)</td>
</tr>
<tr>
<td>HCL (1)</td>
<td>1.10</td>
</tr>
<tr>
<td>ALL (7)</td>
<td>3.33 (1.30–6.70)</td>
</tr>
<tr>
<td>Lymphoid tissue</td>
<td></td>
</tr>
<tr>
<td>Controls (7)</td>
<td>1.18 (0.95–1.40)</td>
</tr>
<tr>
<td>NHL</td>
<td></td>
</tr>
<tr>
<td>DWDLL (5)</td>
<td>1.06 (0.90–1.25)</td>
</tr>
<tr>
<td>NPDLL (14)</td>
<td>1.34 (0.42–4.10)</td>
</tr>
<tr>
<td>DPDDL (6)</td>
<td>1.67 (0.80–3.30)</td>
</tr>
<tr>
<td>DHL (16)</td>
<td>2.25 (0.50–3.80)</td>
</tr>
<tr>
<td>HD (8)</td>
<td>1.26 (0.45–3.00)</td>
</tr>
</tbody>
</table>

$^a$ Numbers in parentheses, number of samples.
$^b$ Mean CTP synthetase activity.
$^c$ Numbers in parentheses, range of enzyme activities.

**DISCUSSION**

The determination of enzymes in the lymphoproliferative disorders has been largely carried out using cytochemical identification as an ancillary aid in the establishment of the cell origin of the neoplastic clone (16). More recently, it has been established that the activity of a number of purine and pyrimidine enzymes differs substantially among some types of lymphoproliferative disorders (3, 9). Both in vitro and in vivo studies have demonstrated that these biochemical differences are exploitable (8, 10, 20, 27). In this study, the activity of the pyrimidine enzyme CTP synthetase was measured in lymphopoietic disorders, and the enzyme level correlated with histological features and in some types with the immunological characteristics and thymidine kinase activity.

Although the mean CTP synthetase activity in ALL was almost 4-fold the mean control level, the profile of enzyme activity in this category showed considerable heterogeneity. The highest CTP synthetase activity occurred in C-ALL lymphoblasts, but the variation in enzyme levels did not correlate with immunological typing. However, in 3 of 5 examples, a high enzyme...
level was associated with clinical resistance to current antileukemic agents. In this situation, expansion of the dCTP pool as a result of increased CTP synthetase activity could contribute to clinical ara-C resistance (1). CTP synthetase in B-CLL showed a narrow range of activities within that of the control peripheral blood lymphocytes, which is consistent with the well-differentiated appearance and low proliferative rate of B-CLL cells (9). In contrast, T-CLL cells exhibited an increase in CTP synthetase activity, suggesting that these leukemic cells have a greater proliferative rate than do B-CLL cells.

The stepwise increase in mean CTP synthetase activity of the Rappaport NHL types DWDLL, NPDLL, DPDLL, and DHL correlates with the morphological and biological transformation that is expressed in this classification of NHL (2, 16). Clinical relevance for these findings is suggested by the finding of a significant difference between the enzyme activities in favorable histological categories and in the unfavorable DHL type. The narrow range of enzyme activities found in DWDLL was closely similar to the findings in its leukemic counterpart B-CLL, but marked heterogeneity was an outstanding feature of the profile of CTP synthetase activity in the other types of NHL examined. The widest range was found in NPDLL, a type long considered to be relatively homogeneous but one which recent data indicate is heterogeneous, particularly with respect to cell proliferation (6, 7, 9). Among the high enzyme levels in favorable categories were 2 examples of NPDLL and one instance of DPDLL which showed histological progression to the aggressive DHL type. The wide variation found in the CTP synthetase activity of NHL is consistent with the known morphological, immunological, kinetic, biochemical, and clinical heterogeneity of this category (9, 13, 22, 24). The profile of CTP synthetase activity in NHL is very similar to that of thymidine kinase, with the stepwise increase in mean enzyme activity of the Rappaport categories broadly correlating with the known biological aggressiveness of each category (9). This finding is amplified by the data for HD, in which the highest CTP synthetase activities occurred in the most malignant subtype, lymphocyte-depleted HD.

These data suggest that, like rat hepatoma CTP synthetase (25) and human lymphoid thymidine kinase (8, 9), CTP synthetase levels in human malignant lymphomas reflect the proliferative rate of individual tumors and hence clinical aggressiveness. However, the lack of a strong correlation between tumor thymidine kinase and CTP synthetase activities indicates that other as yet undetermined biological factors also influence the intracellular CTP synthetase level.

Inhibition of CTP synthetase activity is thought to be the primary mode of action of the investigational antimetabolite DAU (17). Recent evidence which shows CTP synthetase to be an important contributor to intracellular CTP and dCTP pools (19, 25) and the experimental synergism between DAU and the antileukemic agents 5-azadeoxycytidine (18) and ara-C (14) suggest that there is therapeutic value to be gained in inhibiting CTP synthetase. The considerable heterogeneity documented in the CTP synthetase activity of the various types of lymphoproliferative disorders indicates that the preexisting CTP synthetase level will be a significant determinant of DAU cytotoxicity.

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