Biochemical Pharmacology and Resistance to 2-Chloro-2'-arabino-fluoro-2'-deoxyadenosine, a Novel Analogue of Cladribine in Human Leukemic Cells

Kourosh Lofti, Emma Månsson, Tatjana Spasokoukotskaja, Birgitta Pettersson, Jan Lillemark, Curt Peterson, Staffan Eriksson, and Freidoun Albertioni

Department of Medicine and Care, Clinical Pharmacology [K. L., C. P., B. P.], Faculty of Health Sciences, SE-581 85 Linköping, Sweden; Departments of Clinical Pharmacology [E. M., F. A.] and Clinical Oncology [J. L.], Karolinska Hospital, SE-171 76 Stockholm, Sweden; Department of Medical Chemistry, Molecular Biology and Pathobiochemistry, Semmelweis University of Medicine, H-1444 Budapest, Hungary [T. S.]; and Department of Veterinary Medical Chemistry, Swedish University of Agricultural Sciences, The Biomedical Center, SE-751 85 Uppsala, Sweden [S. E.]

ABSTRACT

The objective of the present study was to investigate the biochemical pharmacology of 2-chloro-2'-arabino-fluoro-2'-deoxyadenosine (CAFdA) — a fluorinated analogue of cladribine (2-chloro-2'-deoxyadenosine, Leustatin (CdA)) — with improved acid and metabolic stability — in human leukemic cell lines and in mononuclear cells isolated from patients with chronic lymphocytic leukemia (CLL) and acute myeloid leukemia (AML). We have also made and characterized two cell lines that are not sensitive to the growth inhibitory and cytotoxic effects of CAFdA. Incubation of cells isolated from the blood of CLL and AML patients with various concentrations of CdA or of CAFdA accumulated CdA and CAFdA nucleotides in a dose-dependent manner. A significantly higher rate of phosphorylation to monophosphates was observed for CAFdA than for CdA in cells from CLL patients (n = 14; P = 0.04). The differences in the phosphorylation were even more pronounced for the respective triphosphates in both CLL (n = 14; P = 0.001) and AML (n = 4; P = 0.04) cells. Retention of CAFdA 5'-triphosphate (CAFdATP) was also longer than that for CdA 5'-triphosphate (CdATP) in cells from leukemic patients. The relative efficacy of CAFdA as a substrate for purified recombinant deoxycytidine kinase (dCK), the key enzyme in the activation of nucleoside analogues, was very high and exceeded that of CdA as well as the natural substrate, deoxycytidine, by a factor of 2 and 8, respectively. The Kₚ for CAFdA with dCK was also lower than that for CdA, as measured in crude extracts from the human acute lymphoblastic leukemia cell line CCRF-CEM and the promyelocytic leukemia cell line HL60. Acquired resistance to CAFdA in HL60 and in CCRF-CEM cell lines was directly correlated to the decreased activity of the nucleoside phosphorylating enzyme, dCK. Resistant cells also showed a considerable degree of cross-resistance to analogues that were activated by dCK. These observations demonstrated that dCK phosphorylates CAFdA more efficiently than CdA. Furthermore, CAFdATP is apparently more stable than CdATP and the mechanisms of resistance to CAFdA are similar to those leading to CdA resistance. These results encourage studies on the clinical effect of CAFdA in lymphoproliferative diseases.

INTRODUCTION

Progress in antileukemia chemotherapy has been achieved partially by the development of new nucleoside analogues with novel mechanisms of action such as Fara-A³ [2F-adenine arabinoside (Fludara)] and CdA [2-chloro-2'-deoxyadenosine (Leustatin); Ref. 1]. CdA is an effective drug for lymphoproliferative diseases, e.g., hairy cell leukemia (2, 3), CLL (4, 5), low-grade non-Hodgkin’s lymphoma (6), and AML (7, 8), as well as in autoimmune disorders and multiple sclerosis (2, 9, 10). One disadvantage of CdA is that it has only a 50% oral bioavailability (11) caused by instability at acidic pH and degradation by bacterial nucleoside phosphorylases (12).

CAFdA is a 2'-arabino-fluoro derivative of CdA. The introduction of a fluorine at the 2'-arabino position of CdA has increased its acid stability. The solubility problems associated with the administration of Fara-A and the low oral bioavailability of CdA may be overcome by using CAFdA. We have reported previously (13) that in some patients the plasma chloro-adenine, a hydrolysis product of CdA, exceeded that of the CdA concentration when patients were treated with CdA p.o. ³The abbreviations used are: Fara-A, fludarabine; CdA, 2-chloro-2'-deoxyadenosine; CdATP, CdA 5'-triphosphate; CdAMP, CdA 5'-monophosphate; CLL, chronic lymphocytic leukemia; AML, acute myeloid leukemia; CAFdA, 2-chloro-2'-arabino-fluoro-2'-deoxyadenosine; CAFdAMP, CAFdA monophosphate; CAFdATP, CAFdA triphosphate; dCK, deoxycytidine kinase; dCyd, deoxycytidine; dGK, deoxyguanosine kinase; HPLC, high-pressure liquid chromatography; AUC, area(s) under (the cellular concentration versus time) curve; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.
CAFdA was more stable against deglycosylation by hepatic enzymes as compared with CdA (14).

The mechanism of action of CAFdA has been studied in detail elsewhere (15–17). CAFdA has properties that combine the features of two clinically used drugs, CdA and Fara-A. CAFdA, like CdA and Fara-A, is toxic to nondividing human lymphocytes as well as to rapidly proliferating cells. CAFdA is phosphorylated to its monophosphate mainly by dCK and also by mitochondrial enzyme dGK (18) and, after further cellular phosphorylation, to the final active metabolite CAFdATP. CAFdATP inhibited ribonucleotide reductase to an extent similar to CdATP, and DNA polymerase to an extent similar to Fara-ATP. However, data on metabolism and the mechanism of drug resistance to CAFdA are limited in humans, and, thus far, there is only one report on CAFdA pharmacokinetics in animals (19). A therapeutic effect was shown after a 1-week oral treatment with CAFdA, which eliminated 90% of the CLL cells transplanted into severe combined immunodeficiency mice (20). CAFdA administered p.o. has also been shown to be an effective agent against human colon tumor xenografts (21).

In the present study, we have characterized the intracellular metabolism of CAFdA to its mono-, di-, and triphosphate derivatives in vitro, both in human cell lines and in peripheral blood mononuclear cells isolated from CLL and AML patients. We have also determined the kinetic parameters for dCK using CAFdA as substrate in crude extracts from various human cell lines, as well as with purified recombinant dCK. The results were compared with those obtained with CdA to evaluate the therapeutic effectiveness of CAFdA as a basis for future clinical trials in humans. Moreover, human T-lymphoblastic (CCRF-CEM) and human promyelocytic leukemia (HL60) cell lines were exposed continuously to increasing concentrations of CAFdA over a period of 8–12 months. At intervals of 2 weeks, the concentrations of the drugs were increased by 5 nM. Then, the resistant cell lines were kept at concentrations of 100 nM CAFdA in CCRF-CEM cells (CEM/CAFdA) and 150 nM CAFdA in HL60 (HL60/CAFdA) cells for 2 months. The cell lines maintained stable resistance for at least 20 passages in the absence of drugs. All of the cell lines were kept in suspension culture in exponential growth in RPMI 1640 containing heat-inactivated FCS (10%), penicillin (100 units/ml), streptomycin (100 µg/ml), and l-glutamine (2 mM; all from Life Technologies, Inc., Paisley, United Kingdom) at 37°C in a humidified atmosphere containing 5% CO2 and were routinely tested for Mycoplasma contamination.

The number of cells in G1, S, and G2+M phase of the cell cycle were determined using a PAS II cytometer (Partec, Münster, Germany), which was equipped with a mercury arc lamp. 4,6-diamidino-2-phenylindole was excited at 365 nm and the fluorescence was measured at >435 nm. The multicycle program (Phoenix Flow Systems, San Diego, CA) was used for histogram analysis.

Before each experiment, the cell lines were subcultured in drug-free medium for at least 2 passages and then they were cultured with logarithmic growth (about 0.8–1.3 × 10^6) over a period of 3 days, washed twice with prewarmed RPMI 1640-HEPES, and counted. The cells were resuspended in the same medium to a final count of 1–2 × 10^6 cells/ml.

Cytotoxicity Assay. The cytotoxicity assay was performed as described by Mosmann et al. (22). Briefly, cell lines were suspended at 2 × 10^3 cells/ml, and 100-µl aliquots were dispensed into 96-well round-bottomed microtiter plates that already contained 5 µl of drug dilutions in triplicate. Wells containing no drugs were used as controls, and wells containing no cells were used as blanks. The plates were then incubated at 37°C for 72 h in an atmosphere of 5% CO2. We also investigated whether a continuous or a short incubation of CAFdA might have different efficacies in CCRF-CEM and HL60 cells. The cells were incubated either with 50 nM drug continuously for 120 h or with 300 nM drug for 2 h every 24 h over five days. Afterward, 10 µl of a stock solution of 5 mg/ml MTT (Sigma, St. Louis, MO) was added to each well, and the plates were incubated for an additional 4 h at 37°C. Formazan crystals were dissolved with 100 µl of 10% SDS/10 mM HCl solution overnight at 37°C. Absorbance (A) was measured using an ELISA.

MATERIALS AND METHODS

Leukemic Cell Isolation and Incubation. The study was approved by the ethics committee at Karolinska Hospital, Stockholm, Sweden. Blood from 14 patients with CLL and 4 patients with AML was collected from a peripheral IV access into heparinized tubes. Mononuclear cells were isolated by density gradient centrifugation with Lymphoprep (Nycomed, Oslo, Norway). After washing with PBS, 2 ml of ice-cold distilled water was added to hemolyze the RBCs. After 30 s, 1 ml 2.7% NaCl was added, and the volume was adjusted to 50 ml with PBS and centrifuged. The number of cells in the samples and the median cell volume of the samples was determined by a Coulter Multisizer (Coulter Electronics, Luton, United Kingdom).

Cells were suspended in RPMI 1640 containing 25 mM HEPEES (RPMI 1640-HEPES (Life Technologies, Inc., Paisley, United Kingdom) to get 1 × 10^6 cells/ml; and CdA (from Dr. Zygmunt Kazimierczuk, Foundation for the Development of Diagnostics and Therapy, Warsaw, Poland) or CAFdA (kindly provided by Dr. Howard Cottam, University of California, San Diego, CA) was added to the medium at a concentration 0.05–25 µM. After incubation for 2 h at 37°C, cells were centrifuged, washed twice with ice-cold PBS, and extracted for nucleotide determinations, performed as described in “HPLC Determination of CdA and CAFdA Nucleotides” section. Cells from a CLL patient were incubated with 10 µM CdA or CAFdA for 2 h. After incubation, the cells were centrifuged and resuspended in drug-free RPMI 1640-HEPES. To study the efflux kinetics of CdA and CAFdA, cells were withdrawn at desired intervals, and determinations of CdA and CAFdA nucleotides were performed with HPLC as described below.

Development of Resistant Cell Lines. To delineate the mechanism of resistance to CAFdA, the human T-lymphoblastic (CCRF-CEM) and human promyelocytic leukemia (HL60) cell lines were exposed continuously to increasing concentrations of CAFdA over a period of 8–12 months. At intervals of 2 weeks, the concentrations of the drugs were increased by 5 nM. Then, the resistant cell lines were kept at concentrations of 100 nM CAFdA in CCRF-CEM cells (CEM/CAFdA) and 150 nM CAFdA in HL60 (HL60/CAFdA) cells for 2 months. The cell lines maintained stable resistance for at least 20 passages in the absence of drugs. All of the cell lines were kept in suspension culture in exponential growth in RPMI 1640 containing heat-inactivated FCS (10%), penicillin (100 units/ml), streptomycin (100 µg/ml), and l-glutamine (2 mM; all from Life Technologies, Inc., Paisley, United Kingdom) at 37°C in a humidified atmosphere containing 5% CO2 and were routinely tested for Mycoplasma contamination.

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plate reader (Labsystems Multiscan RC) at the wavelength of 540 nm with reference at 650 nm. Cell survival (CS) was expressed as percentage of control wells:

\[ CS = \frac{\text{mean } A_{\text{heated wells}}}{\text{mean } A_{\text{control wells}}} \times 100\% \]

**HPLC Determination of C'dA and CAF'dA Nucleotides.**

Intracellular C'dA and CAF'dA nucleotides were determined as described previously (23). Briefly, 200 \( \mu \)l of ice-cold 0.4 \( M \) perchloric acid containing 0.08 \( M \) triethylammonium phosphate was added to the cell pellet, and the pH was adjusted to 6.2 by the addition of approximately 100 \( \mu \)l of ice-cold 1.2 \( M \) potassium hydroxide-0.4 \( M \) ammonium dihydrogen-phosphate. The extract was centrifuged at 10,000 \( \times g \) for 5 min. at 4°C, and the final volume of the supernatant was measured. Aliquots of the supernatants were analyzed immediately by HPLC or stored at \(-20^\circ\)C. The column was an Ultrasphere ODS (250 \( \times \) 4.6 mm, 5 \( \mu M \), Beckman Instruments Fullerton, CA) equipped with the Guard-Pak precolumn (\( \mu \)Bondapak C18; Millipore, Milford, MA). The mobile phase consisted of triethylammonium phosphate buffer [TEAP, 0.08 \( M \) (pH 6.1)] and methanol (89:11, v/v), and the flow-rate was 1.8 –2 ml/min. The temperature of the autosampler was kept at 8°C. The HPLC system consisted of a Milton Roy CM 4000 pump, a variable-wavelength detector (LDC Division, Riviera Beach, FL) and a CMA-240 autosampler (Carnegie Medicine, Stockholm, Sweden). C'dATP was synthesized by Sierra Bioresearch (Tucson, AZ) and provided by Dr. William Plunkett (Department of Clinical Investigation, The University of Texas, Houston, TX). The retention times for CdAMP, CdATP, CAFdAMP, and CAFdATP were 12, 15.5, 18.5, and 25 min., respectively. The absorbances were detected at a wavelength of 265 nm; the concentrations, expressed in \( M \), were determined by using \[ \frac{\text{mean } A_{\text{heated wells}}}{\text{mean } A_{\text{control wells}}} \times 100\% \]

**Fig. 1** Dose-dependent accumulation of C'dAMP (●), C'dATP (○), CAF'dAMP (■), and CAF'dATP (□) in the cells of patients with CLL (A, \( n = 14 \)) and AML (B, \( n = 4 \)). Peripheral blood leukemic cells were incubated with the indicated concentrations of CAF'dA and C'dA for 2 h. They were washed, extracted, and analyzed for C'dA and CAF'dA nucleotide content as described in “Materials and Methods.” The accumulation of C'dA 5'diphosphate and CAF'dADP represented less than 5–10% of the total nucleotides and could not be quantitated adequately. The cellular accumulation rate is expressed as pmol per h.
Besides this, in AML patients, the triphosphate:monophosphate ratios of nucleoside analogues were analyzed by paired t test. The differences were calculated according to a commercially available program (Intuitive Software for Science, San Diego, CA).

Western Blotting. The amount of dCK protein was determined in HL60 and CCRF-CEM cell extracts by Western blot. A peptide anti-dCK antibody was used as previously described (27). Approximately 40 μg of crude cell extract and 2 μg of purified recombinant dCK were analyzed, and the protein bands were detected by the enhanced chemiluminescence method as described by the suppliers (Amersham).

Protein Determination. The protein content in cell extracts was determined according to the method of Lowry et al. (28; DC protein assay, Bio-Rad Laboratories, CA).

Kinetic and Statistical Calculations. The areas under the cellular concentration versus time curve (AUC) from time 0 up to the time that the last measured CdA and CAFdA nucleotides concentration were calculated according to the linear trapezoidal rule. Statistics were calculated using StatView software (Abacus Concepts, Berkeley, CA). The differences between CdA and CAFdA nucleotides were analyzed by paired t test analysis and between CLL and AML patients by unpaired t test. The areas under the cellular concentration versus time curve (AUC) were calculated for at least two separate experiments performed in triplicate.

RESULTS

Comparison of Cellular Accumulation and Retention of CAFdA and CdA Metabolites in Leukemic Cells. Analysis of the nucleotide fractions by HPLC after in vitro incubation of leukemic cells from CLL (n = 14) and AML (n = 4) patients for 2 h, with increasing concentrations of CdA and CAFdA, showed a concentration-dependent accumulation of CdA and CAFdA nucleotides (Fig. 1, A and B).

In cells from CLL patients, the initial and maximal velocities of CAFdAMP and CAFdATP accumulation were significantly elevated compared to those for CdAMP and CdATP (Fig. 1A). In CLL cells at 1-μM exogenous concentration of analogues, the mean ratio of CAFdAMP:CdAMP concentrations was 1.6 ± 0.5 (P < 0.05), and an even higher CAFdATP:CdATP ratio was achieved (4.3 ± 3.5; P = 0.001). In cells from AML patients, the kinetic of CAFdA and CdA monophosphates accumulation showed practically no differences, whereas CAFdATP concentrations considerably exceeded those of CdATP at all of the concentrations of analogues (P = 0.04; Fig. 1B). Besides this, in AML patients, the triphosphate:monophosphate ratios, as well as the absolute triphosphate concentrations of both analogues were 3- to 4-fold higher compared with CLL patients (Fig. 1, A and B).

The retention of CAFdA and CdA nucleotides accumulated in CLL cells after a 2-h exposure to 10-μM concentrations of analogues was followed up to 48 h after the exposure. The decay of CdA mono- and triphosphates in cells after resuspension in drug-free medium was biphasic with a prolonged terminal phase for CAFdATP. The intracellular half-life of CAFdATP was longer than that for CdATP (1/2, 7.3 and 4.3 h, respectively). The CAFdAMP exposure, expressed as the area under the nucleotide concentration versus time curve (AUC) to the CLL cells was higher for CAFdA (302 μM × h) when compared with corresponding values of CdA (117 μM × h). The differences in the AUC for triphosphates were even more pronounced (250 and 66 μM × h for CAFdATP and CdATP, respectively). More than 90% of the CdATP and CAFdATP was eliminated concentrations of nucleoside in a total volume of 50 μl. The phosphorylated products were separated by TLC and quantitated as described previously (26). Vmax and Km were calculated using a commercially available program, GraphPad PRISM Version 2.0 (Intuitive Software for Science, San Diego, CA).

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Table 1 Kinetic parameters of nucleoside analogues with dCK by purified recombinant dCK using phosphoryl transfer assay

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
<th>Km (μM)</th>
<th>Vmax</th>
<th>Relative efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purified dCK</td>
<td>dCyd</td>
<td>6.3 ± 2.5</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>CdA</td>
<td>5.8 ± 0.9</td>
<td>2.1 ± 0.1</td>
<td>232</td>
</tr>
<tr>
<td></td>
<td>CAFdA</td>
<td>3.3 ± 0.6</td>
<td>4.0 ± 1.0</td>
<td>760</td>
</tr>
</tbody>
</table>

*Data are expressed as mean ± SD of at least two separate experiments performed in triplicate.

10 mM DTT, 0.5 mg/ml BSA, dCK, and various concentrations of nucleoside in a total volume of 50 μl. The phosphorylated products were separated by TLC and quantitated as described previously (26). Vmax and Km were calculated using a commercially available program, GraphPad PRISM Version 2.0 (Intuitive Software for Science, San Diego, CA).

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from the cell within 48 h of incubation in drug-free medium (data not shown).

Phosphorylation of CAFdA and CdA by Pure Recombinant dCK and by the Kinases in Crude Cell Extracts. An explanation for the higher intracellular levels of CAFdATP can be found in the more efficient phosphorylation of CAFdA by dCK as compared with CdA. Table 1 shows the $K_m$ and relative $V_{max}$ values for CdA, CAFdA, and natural substrate, dCyd, with recombinant dCK using the phosphoryl transfer assay. CAFdA was three times more efficient than CdA and showed nearly 8-fold higher efficiency than the natural substrate dCyd. In crude extracts of CCRF-CEM cells, the $K_m$ values—measured with radiolabeled dCyd, CdA, and CAFdA—were 2.5, 3.4, and 2.3 $\mu M$, respectively. In extracts of HL60 cells, the $K_m$ values were 2.0, 2.9, and 1.4 $\mu M$, respectively. Thus both the recombinant dCK and the enzyme from leukemic cells had higher affinity to CAFdA, than to CdA.

Sensitivity and Resistance to CAFdA in CCRF-CEM and HL60 Cells. Human leukemia cell lines growing in suspension cultures were incubated with different concentrations of CAFdA. The concentrations of analogue-inhibiting cell growth by 50% ($IC_{50}$) in CCRF-CEM and HL60 were 0.05 $\pm$ 0.02 $\mu M$ and 0.04 $\pm$ 0.00 $\mu M$, respectively, as measured after 72 h (Fig. 2, A and B). We found a similar toxicity toward CCRF-CEM and HL60 when cells were exposed to CAFdA as continuous treatment (50 nM for 120 h) or short treatment (300 nM for 2 h every 24 h for 5 days; data not shown). The choice of CAFdA concentration was based upon the concentration achievable clinically in plasma in patients treated with CdA as continuous infusion (steady state level) or as short infusions (maximum concentration), respectively (11).

To investigate the molecular mechanisms of resistance to CAFdA, CCRF-CEM and HL60 cells were exposed to CAFdA continuously at concentrations achievable clinically in patients treated with CdA (10–300 nM). Two resistant stable clones designated as CEM/CAFdA and HL60/CAFdA were selected and characterized with respect to growth inhibition and cross-resistance to other antimetabolites and clinically used anticancer drugs. The cells were more than 1000-fold less sensitive to the growth inhibitory and cytotoxic effects of CAFdA relative to

Table 2  Biological and biochemical characteristics of CCRF-CEM and HL60 wild types and CAFdA-resistant cell lines

<table>
<thead>
<tr>
<th></th>
<th>CCRF-CEM/wt</th>
<th>CEM/CAFdA</th>
<th>HL60/wt</th>
<th>HL60/CAFdA</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC$_{50}$, $\mu M$ CAFdA$^a$</td>
<td>0.05</td>
<td>2.65</td>
<td>0.04</td>
<td>5.00</td>
</tr>
<tr>
<td>% in S phase$^a$</td>
<td>51</td>
<td>39</td>
<td>46</td>
<td>43</td>
</tr>
<tr>
<td>Doubling time (h)$^a$</td>
<td>22</td>
<td>27</td>
<td>34</td>
<td>30</td>
</tr>
<tr>
<td>Cell size, fl$^a$</td>
<td>530</td>
<td>570</td>
<td>540</td>
<td>560</td>
</tr>
<tr>
<td>1-h generation of CAFdAMP/CAFdATP, $\mu M$$^a$</td>
<td>53.2/54.5</td>
<td>0.6/0.4</td>
<td>26.6/9.1</td>
<td>1.7/0.4</td>
</tr>
<tr>
<td>dCK, dCyd as substrate$^b$</td>
<td>42.4 $\pm$ 1.5</td>
<td>0.3 $\pm$ 0.1</td>
<td>22.5 $\pm$ 1.4</td>
<td>5.3 $\pm$ 0.2</td>
</tr>
<tr>
<td>dCK, CdA as substrate$^b$</td>
<td>48.4 $\pm$ 1.9</td>
<td>11.7 $\pm$ 0.1</td>
<td>30.0 $\pm$ 0.8</td>
<td>10.6 $\pm$ 1.0</td>
</tr>
<tr>
<td>dGK, CdA as substrate in excess of dCyd$^b$</td>
<td>6.2 $\pm$ 0.8</td>
<td>11.7 $\pm$ 0.5</td>
<td>2.6 $\pm$ 0.2</td>
<td>1.5 $\pm$ 0.4</td>
</tr>
<tr>
<td>Thymidine kinase$^b$</td>
<td>43.4 $\pm$ 4.0</td>
<td>54.8 $\pm$ 9.9</td>
<td>28.1 $\pm$ 5.2</td>
<td>24.0 $\pm$ 2.0</td>
</tr>
</tbody>
</table>

$^a$ Data are expressed as mean of at least two experiments performed in triplicate. The CV within the experiments was less than 15%.

$^b$ Data are presented as mean $\pm$ SD of three separate experiments performed in duplicate. Enzymes were expressed as pmol/10$^6$ cells over 1 min.

Fig. 3  Immunoblot analysis of recombinant dCK and dCK contents in CCRF-CEM wild type (CCRF-CEM/wt) and HL60 wild type (HL60/wt) and the corresponding CAFdA-resistant sublines CEM/CAFdA and HL60/CAFdA, using anti-dCK antibody as described in “Materials and Methods.” Approximately 40 $\mu g$ of crude cell extract and 2 $\mu g$ of purified dCK were analyzed. The protein bands were detected by the enhanced chemiluminescence (Amersham) method.
activity measurements in these cells. The relative activity of dCK measured in CEM and HL60 wild-type cell extracts (Fig. 3). Although the CAFdA-resistant cell line extracts, whereas it was easily assayed and the dCK enzyme dGK was similar to CdA and it was 60% of that of the natural substrate, deoxyguanosine (18). In cells and tissues where dCK is absent, dGK is mostly likely to be the main CdA- and CAFdA-activating enzyme. It is notable that some increment in the relative activity of dGK was observed in the CCRF-CEM mutant cell line (Table 2). These cells were shown to be more sensitive to CdA than to CAFdA (Fig. 2A). Thus, dGK may have a significant role in the cytotoxicity of nucleoside analogues in leukemic cells lacking dCK (29).

ACKNOWLEDGMENTS

We thank Dr. A. Gruber from Karolinska Hospital, Stockholm, Sweden, for providing the patient samples and Dr. J. Wang for performing the assay with recombinant dCK. We are also grateful to M. Pålson and K. Ljungcrantz for skillful technical assistance.
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