Effect of Aldehyde Dehydrogenase Inhibitors on the ex Vivo Sensitivity of Human Multipotent and Committed Hematopoietic Progenitor Cells and Malignant Blood Cells to Oxazaphosphorines

Fred R. Kohn, Gregory J. Landkamer, Carl L. Manthey, Norma K. C. Ramsay, and Norman E. Sladek

ABSTRACT

The ex vivo sensitivity of human multipotent and committed hematopoietic progenitor cells and several cultured human malignant blood cell lines to analogues of "activated" cyclophosphamide, namely, 4-hydroxycyclophosphamide and mafosfamide, and to phosphoramide mustard was quantified with and without concurrent exposure to an inhibitor of aldehyde dehydrogenase activity, namely, disulfiram, cydanamide, diethylidithiocarbamate, or ethylene(2-formylmethyl)phosphinate. Inhibitors of aldehyde dehydrogenase activity potentiated the cytotoxic action of 4-hydroxycyclophosphamide and mafosfamide toward all of the hematopoietic progenitors; they did not potentiate the cytotoxic action of phosphoramide mustard toward these cells. Potentiation of the cytotoxic action of mafosfamide toward cultured human malignant blood cells was minimal. Spectrophotometric assay revealed little NAD-linked aldehyde dehydrogenase activity present in the cultured human tumor cell lines as compared to that found in normal mouse liver or oxazaphosphorine-resistant L1210 cells. Cellular aldehyde dehydrogenases are known to catalyze the oxidation of 4-hydroxycyclophosphamide/aldophosphamide, the major intermediate in cyclophosphamide bioactivation, to the relatively nontoxic acid, carboxyphosphamide. Thus, our findings indicate that (a) human multipotent hematopoietic progenitor cells contain the relevant aldehyde dehydrogenase activity, (b) the relevant activity is retained upon differentiation to progenitors committed to the megakaryocytic/myeloid/monocytic and erythroid lineages, and (c) the relevant activity may be lost or diminished upon transformation of hematopoietic progenitors to malignant cells.

INTRODUCTION

The oxazaphosphorines, of which cyclophosphamide is the prototypic compound, are a clinically important group of antineoplastic and immunosuppressive agents. Recently, analogues of "activated" cyclophosphamide, namely, 4-hydroxycyclophosphamide and mafosfamide, have also shown clinical utility when used ex vivo to purge occult tumor cells from autologous bone marrow samples prior to reinfusion into leukemic patients (1-3). Under physiological conditions, these agents rapidly and spontaneously (without benefit of enzymatic involvement) give rise to 4-hydroxycyclophosphamide/aldophosphamide, the major intermediate in cyclophosphamide bioactivation. Upon β-elimination of acrolein, 4-hydroxycyclophosphamide/aldophosphamide gives rise to phosphoramide mustard, the pharmacologically active metabolite. Alternatively, 4-hydroxycyclophosphamide/aldophosphamide can be oxidized to the relatively nontoxic acid, carboxyphosphamide, thereby decreasing the amount of phosphoramide mustard that can be formed (4-7). In mice at least, this reaction is catalyzed by a minimum of three isozymes of aldehyde dehydrogenase. Thus, all else being equal, target cell sensitivity to the oxazaphosphorines should be inversely proportional to the amount of the relevant aldehyde dehydrogenase activity present in these cells (4-6, 8-12).

Recent ex vivo investigations in our laboratory (10-12) revealed that inhibitors of aldehyde dehydrogenase activity potentiate the cytotoxic action of oxazaphosphorines toward murine day-8 and -12 spleen colony-forming cells, hematopoietic populating cells, multipotential colony-forming cells (CFU-GEMM), and CFU-Mk, but not toward CFU-GM, BFU-E, and CFU-E. These findings suggest that (a) murine pluripotent hematopoietic stem cells contain the relevant aldehyde dehydrogenase activity, (b) this activity is retained upon differentiation to progenitors committed to the megakaryocytic lineage, and (c) this activity is lost upon differentiation to progenitors committed to the granulocytic/myeloid and erythroid lineages. These findings, and the observation that certain murine, oxazaphosphorine-sensitive tumor cells do not contain the relevant activity (6, 9), indicate that differential aldehyde dehydrogenase activity contributes significantly to the relatively favorable margin of safety exhibited by the oxazaphosphorines.

The preceding hypothesis is based on data generated in a mouse model. The present investigation is a first attempt at testing this hypothesis in humans. Thus, experiments were designed to determine (a) whether human multipotent hematopoietic stem cells (CFU-Mix) contain the relevant aldehyde dehydrogenase activity, (b) if so, whether this activity is lost or retained upon differentiation to progenitors committed to the megakaryocytic/myeloid and erythroid lineages, and (c) whether the relevant activity is lost/regained upon transformation of human hematopoietic precursors to various neoplastic blood cells.

MATERIALS AND METHODS

Materials. Mafosfamide [2-[bis-(2-chloroethyl)-amino]-4-(2-sulfoethylthio)-tetrahydro-2H,1,3,2-oxazaphosphorine-2-oxide cyclohexylamine salt] and 4-hydroxycyclophosphamide were kindly supplied by Dr. P. Hilgard (Asta-Werke AG, Bielefeld, Federal Republic of Germany). Phosphoramide mustard-cyclohexylamine, melphalan HCl, ethylphenyl(2-formylmethyl)phosphinate, and conditioned medium from phytohemagglutinin-stimulated human peripheral blood leukocytes were kindly supplied by L. H. Kedda (Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD), Dr. G. M. Lyon, Jr. (Burroughs Wellcome & Co., Research Triangle Park, NC), Dr. L. A. Cates (College of Pharmacy, University of Houston, Houston, TX), and Dr. F. Uckun (Department of Therapeutic Radiology, University of Minnesota, Minneapolis, MN), respectively. Methylcellulose (A4M premium grade; 4000 cps) was kindly supplied by the Dow Chemical Co., Midland, MI. M-Platinum II diamine dichloride, disulfiram, diethylidithiocarbamate, pyridoxal phosphate were kindly supplied by the Dow Chemical Co., Midland, MI. cis-Platinum II diamine dichloride, disulfiram, diethylidithiocarbamate, pyridoxal hydrochloride, cydanamide, 2-mercaptoethanol, Histopaque-1077, and NAD were purchased from the Sigma Chemical Co., St. Louis, MO.

Received 11/19/86; revised 3/17/87; accepted 3/20/87.

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1 This work is supported by USPHS grant CA 21737. A description of parts of this investigation has appeared in abstract form (33).

2 To whom requests for reprints should be addressed, at the Department of Pharmacology [F. R. K., G. J. L., C. L. M., N. E. S.], and Department of Pediatrics [N. K. C. R.], University of Minnesota Medical School, Minneapolis, Minnesota 55455.

3 The abbreviations used are: CFU-GEMM, colony-forming unit, granulocytoi/myeloid/megakaryocytes; CFU-M, colony-forming unit, megakaryocytes; CFU-GM, colony-forming unit, granulocytic/myeloid; BFU-E, burst-forming unit, erythroid; CFU-E, colony-forming unit, erythroid; CFU-Mix, colony-forming unit, granulocytic/erythroid/megakaryocytes, with or without megakaryocytes; IMDM, Iscove's modified Dulbecco's medium; IMDM/FBS, Iscove's modified Dulbecco's medium supplemented with 10% fetal bovine serum.
Horse serum, RPMI 1640 medium, IMDM, and gentamicin were purchased from the Grand Island Biological Co., Grand Island, NY. Acetaldehyde, human urinary step I erythropoietin, and fetal bovine serum were purchased from the Aldrich Chemical Co., Milwaukee, WI, the Terry Fox Laboratories, Vancouver, British Columbia, Canada, and the Hyclone Laboratories, Logan, UT, respectively.

All drugs were dissolved in drug-exposure medium, *vide infra*, with the exception of disulfiram which was dissolved in 95% ethanol. All drug solutions, except for those containing disulfiram, were sterilized by passage through 0.22-μm Millipore filters; all were used within 30 min of preparation and were kept on ice prior to their use. At the concentrations used, ethanol itself was not cytotoxic and did not alter the sensitivity of hematopoietic progenitor cells to the oxazaphosphorines. Drug-exposure medium was a phosphate-buffered saline-based solution, pH 7.4, prepared as previously described (10).

Animals. DBA/2 mice were obtained from the University of Minnesota Mouse Colony. Animals were housed in plastic cages with filtered lids and were given standard laboratory food and water *ad lib.* Female mice, aged 8 to 12 weeks (18 to 22 g), were used as liver donors.

Human Tumor Cell Lines. HPB-Null, MOLT-4, and Raji cells were a generous gift from Dr. T. W. LeBien (Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN). K-562 and KG-1 cells were generous gifts from Dr. D. A. Vallera (Department of Therapeutic Radiology, University of Minnesota, Minneapolis, MN) and Dr. B. C. Bostrom (Department of Pediatrics, University of Minnesota, Minneapolis, MN), respectively. U-937 and HL-60 cells were purchased from the American Type Culture Collection, Rockville, MD. The surmised ontogenesis of these cell lines is presented in Fig. 1. All cell lines were grown in static suspension culture at 37°C in RPMI 1640 culture medium supplemented with 10% horse serum and a humidified atmosphere of 5% CO₂ in air; except for HL-60, all grew exponentially.

Mouse Tumor Cell Lines. Cultured L1210 cells, sensitive (L1210/0) and resistant to oxazaphosphorines specifically (L1210/OAP), were obtained from the Southern Research Institute, Birmingham, AL through the courtesy of Drs. R. F. Struck and L. J. Wilkoff. Culture conditions and growth conditions were as described previously (9).

Preparation of Bone Marrow Cell Suspensions. Bone marrow cells were obtained by aspiration from the iliac crest of healthy adult volunteers who gave informed written consent. Preservative-free heparin was added as an anticoagulant. Aspirates were centrifuged for 10 min at 800 x g and buffy-coat cells were collected and diluted in IMDM/FBS. Aliquots were layered over a Ficoll gradient (Histopaque-1077) and centrifuged (800 x g for 30 min); buoyant interphase mononuclear cells were collected, washed once with IMDM/FBS, and resuspended in IMDM/FBS. The suspended cells were then transferred to Corning 25-

Fig. 1. Schematic representation of the ontogenesis of several human malignant blood cell lines. *CFU-BL*, colony-forming unit, blast cell; *PRE B*, B-lymphocyte precursor; *PRE T*, T-lymphocyte precursor.
Sensitivity of Hematopoietic Progenitors to Oxazaphosphorines

Table 1  Sensitivity of human hematopoietic progenitor cells to cytotoxic agents with and without concurrent exposure to inhibitors of aldehyde dehydrogenase activity

<table>
<thead>
<tr>
<th>Donor</th>
<th>Drug Combination</th>
<th>CFU-Mix</th>
<th>CFU-Mk</th>
<th>CFU-GM</th>
<th>BFU-E</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mafosfamide</td>
<td>15 (12-19)*</td>
<td>14 (11-18)</td>
<td>21 (19-22)</td>
<td>18 (16-21)</td>
</tr>
<tr>
<td></td>
<td>Mafosfamide + cyanamide</td>
<td>3 (3-3)</td>
<td>4 (2-8)</td>
<td>8 (7-8)</td>
<td>4 (2-7)</td>
</tr>
<tr>
<td>1*</td>
<td>Mafosfamide</td>
<td>19 (12-27)</td>
<td>17 (11-25)</td>
<td>20 (19-20)</td>
<td>21 (17-26)</td>
</tr>
<tr>
<td></td>
<td>Mafosfamide + disulfiram</td>
<td>5 (3-7)</td>
<td>4 (3-6)</td>
<td>9 (8-10)</td>
<td>6 (3-12)</td>
</tr>
<tr>
<td>2</td>
<td>4-HPCP*</td>
<td>20 (18-22)</td>
<td>18 (14-24)</td>
<td>20 (17-24)</td>
<td>20 (18-23)</td>
</tr>
<tr>
<td></td>
<td>4-HPCP + disulfiram</td>
<td>5 (4-8)</td>
<td>3 (3-4)</td>
<td>4 (4-5)</td>
<td>4 (3-6)</td>
</tr>
<tr>
<td>3</td>
<td>Phosphoramidemustard</td>
<td>119 (89-157)</td>
<td>105 (77-145)</td>
<td>141 (119-166)</td>
<td>120 (95-152)</td>
</tr>
<tr>
<td></td>
<td>Melphalan</td>
<td>0.65 (0.51-0.83)</td>
<td>0.55 (0.46-0.66)</td>
<td>0.78 (0.66-0.93)</td>
<td>0.70 (0.58-0.86)</td>
</tr>
<tr>
<td></td>
<td>cis-Platinum</td>
<td>60 (50-73)</td>
<td>48 (39-60)</td>
<td>58 (52-64)</td>
<td>69 (60-78)</td>
</tr>
<tr>
<td>5</td>
<td>Phosphoramidemustard + cyanamide</td>
<td>141 (74-269)</td>
<td>124 (104-147)</td>
<td>206 (169-252)</td>
<td>149 (78-283)</td>
</tr>
<tr>
<td>4</td>
<td>Phosphoramidemustard</td>
<td>120 (85-170)</td>
<td>109 (86-137)</td>
<td>183 (141-238)</td>
<td>90 (73-112)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, 95% confidence interval.
* Second bone marrow sample taken 7 (donor 1) or 3 (donor 2) weeks later.
* 4-HPCP, 4-hydroperoxycyclophosphamide.

RESULTS

The sensitivity of human hematopoietic progenitor cells to mafosfamide, 4-hydroperoxycyclophosphamide, phosphoramidemustard, melphalan, and cis-platinum was quantified following ex vivo exposure of bone marrow cells to these agents in the absence or presence of a known inhibitor of aldehyde dehydrogenase activity, namely, disulfiram (18) or cyanamide (19) (Table 1). Representative concentration-response curves are shown in Fig. 2. Attention is called to several aspects of the results. In the absence of concurrent exposure to an inhibitor of aldehyde dehydrogenase activity, the four progenitor populations were approximately equisensitive to any given agent. For any given drug or drug combination, donor population variability was minimal when sensitivity of each of the progenitor populations to mafosfamide (donors 1 and 2), 4-hydroperoxycyclophosphamide (donors 3 and 4), or phosphoramidemustard (donors 2 and 5) was determined. Moreover, sensitivity to drugs on the part of each of the progenitor cell populations in marrow samples taken 7 weeks apart from one individual (donor 1) was essentially identical. Also of interest is the finding that mafosfamide and 4-hydroperoxycyclophosphamide were approximately equipotent with regard to their toxicity toward the hematopoietic progenitors. Disulfiram and cyanamide each potentiated the cytotoxic action of the oxazaphosphorines toward all of the progenitors assayed; however, the sensitivity of CFU-Mix, CFU-Mk, and BFU-E to the oxazaphosphorines was increased 3- to 6-fold when an inhibitor of aldehyde dehydrogenase activity was included during drug exposure, whereas, in four of five experiments, the sensitivity of CFU-GM to the oxazaphosphorines was only increased 2- to 3-fold in the presence of inhibitor. In contrast, cyanamide did not potentiate the cytotoxic action of phosphoramidemustard toward any of the progenitors.

Aldehyde oxidase is also known to catalyze the oxidation of 4-hydroxycyclophosphamide/aldophosphamide to carboxyphosphamide (20). Pyridoxal is a known substrate for aldehyde oxidase (21) and is therefore expected to competitively inhibit the oxidation of other aldehydes catalyzed by this enzyme. It
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Fig. 2. Ex vivo sensitivity of human multipotent hematopoietic progenitor cells (CFU-Mix) to mafosfamide with or without disulfiram. Nonadherent, light density mononuclear bone marrow cells (Table 1, donor 1), were incubated in the absence (O) or presence (•) of disulfiram (10 mM) for 60 min at 37°C. Mafosfamide or vehicle was added and incubation was continued at 37°C for an additional 30 min. Immediately following drug exposure, cells were harvested and the number of surviving cells was determined via the back-extrapolation assay as described in "Materials and Methods." Acetaldehyde (4.0 mM) was used as the substrate. There was no detectable activity in soluble or particulate fractions obtained from any of the human tumor cell lines or from murine L1210/OAP tumor cells when aldophosphamide (70 μM) was used as the substrate.

The sensitivity of various cultured human malignant blood cells (Fig. 1) to mafosfamide was also quantified in the absence or present of known [cyanamide, diethyldithiocarbamate (18)] or suspected [ethylphenyl(2-formylmethyl)phosphinate (9, 10)] inhibitors of aldehyde dehydrogenase activity; only minimal potentiation was observed indicating the presence of little or the relevant aldehyde dehydrogenase activity in these cells (Table 2). The cytotoxic action of phosphoramide mustard toward K-562 cells was not potentiated by any of the inhibitors (data not presented). Previously, we have reported that inhibitors of aldehyde dehydrogenase activity only minimally, if at all, potentiate the cytotoxic action of the oxazaphosphorines toward oxazaphosphorine-sensitive mouse L1210 cells whereas they markedly potentiate the cytotoxic action of these agents toward oxazaphosphorine-resistant mouse L1210/OAP cells (9).

A spectrophotometric assay was used to directly quantify the amount of NAD-linked aldehyde dehydrogenase activity present in the soluble and particulate fractions of the human tumor cell lines, oxazaphosphorine-sensitive (L1210/0) and -resistant (L1210/OAP) murine L1210 cell lines, and mouse liver (Table 2). Substantial activity was found in the soluble fraction of L1210/OAP cells; it compared favorably to that found in the soluble and particulate fractions of mouse liver. In contrast, activity was minimal or absent in the soluble and particulate fractions of human tumor, and mouse L1210/0, cells; it was also minimal in the particulate fraction of L1210/OAP cells. Hilton (6) and Hilton and Colvin (8) reported similar findings.

Inasmuch as the findings of studies in which aldehyde dehydrogenase activity was directly determined are in qualitative agreement with those in which this activity was indirectly estimated; i.e., by means of probes, namely, inhibitors of aldehyde dehydrogenase activity, the validity of using the latter to estimate aldehyde dehydrogenase activity, necessary when the cell population of interest cannot (easily) be separated from other cell populations, is established.

Table 2. Aldehyde dehydrogenase activity in cultured human and mouse tumor cell lines and the effect of aldehyde dehydrogenase inhibitors on the sensitivity of these cells to mafosfamide

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Species</th>
<th>Classification</th>
<th>Differentiation stage</th>
<th>Doubling time (h)</th>
<th>H2O</th>
<th>DDTC</th>
<th>Cyanamide</th>
<th>EPP</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPB-Null</td>
<td>ALL</td>
<td></td>
<td>Pre-B blast</td>
<td>27</td>
<td>7.5</td>
<td>6.3</td>
<td>5.8</td>
<td>5.4</td>
</tr>
<tr>
<td>MOLT-4</td>
<td>ALL</td>
<td></td>
<td>T-blast II</td>
<td>21</td>
<td>20</td>
<td>15</td>
<td>16</td>
<td>25</td>
</tr>
<tr>
<td>Raji</td>
<td>Burkitt's lymphoma</td>
<td></td>
<td>B-blast I</td>
<td>16</td>
<td>35</td>
<td>27</td>
<td>32</td>
<td>25</td>
</tr>
<tr>
<td>U-937</td>
<td>Human</td>
<td>Histiocytic lymphoma</td>
<td>Monoblast</td>
<td>21</td>
<td>85</td>
<td>55</td>
<td>69</td>
<td>37</td>
</tr>
<tr>
<td>KG-1</td>
<td>AML</td>
<td></td>
<td>Premyeloid blast</td>
<td>27</td>
<td>77</td>
<td>32</td>
<td>43</td>
<td>ND</td>
</tr>
<tr>
<td>HL-60</td>
<td>AML</td>
<td></td>
<td>Promyelocyte</td>
<td>ND</td>
<td>40</td>
<td>36</td>
<td>40</td>
<td>ND</td>
</tr>
<tr>
<td>K-562</td>
<td>CML</td>
<td></td>
<td>Preerythroid blast</td>
<td>21</td>
<td>60</td>
<td>45</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>L1210/0</td>
<td>Mouse</td>
<td>Lymphocytic leukemia</td>
<td></td>
<td>9</td>
<td>13</td>
<td>36</td>
<td>36</td>
<td>40</td>
</tr>
<tr>
<td>L1210/OAP</td>
<td>Mouse</td>
<td>Lymphocytic leukemia</td>
<td></td>
<td>9</td>
<td>164</td>
<td>36</td>
<td>36</td>
<td>11</td>
</tr>
</tbody>
</table>

* Soluble and particulate fractions were prepared from tumor cells, and NAD-linked aldehyde dehydrogenase activity was determined spectrophotometrically as described in "Materials and Methods." Acetaldehyde (4.0 mM) was used as the substrate. There was no detectable activity in soluble or particulate fractions obtained from any of the human tumor cell lines or from murine L1210/OAP tumor cells when aldophosphamide (70 μM) was used as the substrate.

LC99 concentration of drug required to effect a 99% cell kill; DDTC, diethyldithiocarbamate; EPP, ethylphenyl(2-formylmethyl)phosphinate; ALL, acute lymphocytic leukemia; AML, acute myelogenous leukemia; CML, chronic myelogenous leukemia; ND, not determined.

** LC99 values could not be calculated when L1210/0 were incubated with mafosfamide in the presence of inhibitors of aldehyde dehydrogenase activity since complete concentration-response curves were not determined. However, at single concentrations of mafosfamide, potentiation by such inhibitors was nonexistent or minimal (9).

Alternatively, 51 nmol/min/mg protein. This compares to 32 nmol/min/mg protein for mouse hepatic soluble fraction. When aldophosphamide (70 μM) was used as the substrate, rates were 27 and 31 nmol/min/mg protein for the soluble fractions of mouse liver and L1210/OAP cells, respectively.

Alternatively, 0.5 nmol/min/mg protein for mouse hepatic particulate fraction. When aldophosphamide (70 μM) was used as the substrate, the rate was 4.8 nmol/min/mg protein for the particulate fraction of mouse liver, there was no detectable activity in the particulate fraction of L1210/OAP cells.
DISCUSSION

The results of the present investigation indicate that human multipotent hematopoietic progenitor cells (CFU-Mix) contain the aldehyde dehydrogenase activity that is capable of catalyzing the "detoxification" of 4-hydroxyxycloclophosphamide/aldophosphamide, and that this activity is retained upon differentiation to progenitor cells committed to the megakaryocytoid, granulocytoid/monocytoid, and erythroid lineages. This differs somewhat from what has previously been found for the murine hematopoietic system (10–12). In the mouse, the relevant aldehyde dehydrogenase activity is apparently lost upon differentiation of multipotent stem cells to progenitors committed to the granulocytoid/monocytoid and erythroid lineages (Fig. 3).

The finding that human BFU-E contain aldehyde dehydrogenase activity is not surprising since it is known that the end product of human erythropoiesis, namely, mature erythrocytes, also contain this activity (23). On the other hand, circulating human platelets apparently do not contain aldehyde dehydrogenase activity (24), indicating that this activity is lost either during the differentiation of CFU-Mk into megakaryocytes or during the formation of platelets from megakaryocytes. Not known is whether differentiated human granulocytes or monocytes contain aldehyde dehydrogenase activity.

Human peripheral blood lymphocytes that proliferate in response to phytohemagglutinin (presumably, T-cells) apparently do not contain significant amounts of aldehyde dehydrogenase activity (24). It has also been reported that mitogen- and alloantigen-induced proliferative responses of human peripheral T-lymphocytes are effectively inhibited by lower concentrations of 4-hydroperoxycyclophosphamide than are required for inhibition of proliferation of human bone marrow CFU-GM (25). These findings, coupled with those of the present investigation, suggest that differential aldehyde dehydrogenase activity may account for the differential sensitivity of T-lymphocytes and hematopoietic precursor cells to oxazaphosphorines.

It is not known whether the differentiated end products of murine hematopoiesis and lymphopoiesis contain aldehyde dehydrogenase activity. This is of interest in light of our finding that differences exist between the murine and human systems with regard to the presence of aldehyde dehydrogenase activity in various hematopoietic progenitors.

Recently, an assay has been developed for the quantification of a class of human hematopoietic precursor cells present in bone marrow that are capable of forming undifferentiated blast cells colonies in semisolid culture (26). It has been suggested (26) that these blast cell colony-forming cells are pluripotent and even more primitive than CFU-Mix, even though the latter may be pluripotent as well, since (a) they are capable of self-renewal, (b) they give rise to CFU-Mix, and (c) they resemble murine spleen colony-forming cells in that they do not express Ia-like (HLA-DR) antigen (26, 27); this distinguishes them from human CFU-Mix and known committed hematopoietic progenitors since HLA-DR is expressed in these cells (26, 28). The blast cell colony-forming cells are, as compared to human CFU-GM, markedly less sensitive to 4-hydroperoxycyclophosphamide (29). Our studies and those of others (30) indicate that human CFU-Mix and CFU-GM are approximately equisensitive to this agent. We have also shown that human CFU-Mix contain substantial amounts of the relevant aldehyde dehydrogenase activity. Thus, it may be that the blast cell colony-forming cells are, as compared to CFU-Mix, substantially less sensitive to 4-hydroperoxycyclophosphamide and other oxazaphosphorines because they contain even greater amounts of the relevant aldehyde dehydrogenase activity. If correct, this may explain why (a) successful hematopoietic reconstitution was achieved in myeloablated leukemic patients given autologous marrow that had been removed prior to myeloablation and had subsequently been treated with a concentration of 4-hydroperoxycyclophosphamide sufficient to eliminate virtually all detectable CFU-Mk and committed hematopoietic progenitors of myeloid and lymphoid origin that we tested contained little or none of the relevant aldehyde dehydrogenase activity. In any case, the blast cell colony-forming cells are, as compared to CFU-Mix, substantially less sensitive to 4-hydroperoxycyclophosphamide and other oxazaphosphorines because they contain even greater amounts of the relevant aldehyde dehydrogenase activity.

The seven cultured human tumor cell lines of myeloid and lymphoid origin that we tested contained little or none of the relevant aldehyde dehydrogenase activity. In those cases where measurements were made in normal progenitor cells from which the malignant cells presumably originated (Fig. 1), substantially greater amounts of the relevant aldehyde dehydrogenase activity were found. These observations suggest that aldehyde dehydrogenase activity may be lost upon neoplastic transformation. It is possible that the lack of aldehyde dehydrogenase activity in the malignant cells is an artifact due to a change in phenotype following adaptation of the cells to culture. Moreover, some of these cells are known to differentiate in culture, and differentiation may bring about a loss of aldehyde dehydrogenase activity. This is of interest in light of our finding that differences exist between the murine and human systems with regard to the presence of aldehyde dehydrogenase activity in various hematopoietic progenitors.

Our finding that critical normal human cells, i.e., hematopoietic...
Sensitivity of hematopoietic progenitors to oxazaphosphorines

Hematopoietic progenitor cells, contain substantial amounts of the relevant aldehyde dehydrogenase activity, whereas at least some human tumor cells of myeloid and lymphoid origin contain little or none of this activity, suggests that aldehyde dehydrogenase activity contributes significantly to the favorable margin of safety exhibited by the oxazaphosphorines when used in vivo as antileukemic agents and ex vivo to purge residual tumor cells from autologous bone marrow samples. In mice, the phenotypic basis of acquired leukemia cell resistance to oxazaphosphorines can be an increase in aldehyde dehydrogenase activity (6, 9). Thus, it is possible to progress from cells containing aldehyde dehydrogenase activity, namely, normal hematopoietic precursor cells, to cells lacking this activity, namely, oxazaphosphorine-sensitive malignant cells of lymphoid origin, to malignant cells that again contain this activity. The basis for these changes in phenotype, i.e., whether the critical event occurs at the level of the genome, transcription, translation, or posttranslation, is not known. It may be that the genetic apparatus required for the synthesis of the relevant aldehyde dehydrogenase isozyme(s) is simply repressed in malignant cells and that depression allows for the resumption of synthesis and thus resistance. Acquired resistance to the oxazaphosphorines is often observed in humans as well. Whether it is occasionally/inevitably due to increased aldehyde dehydrogenase activity is not known.

There are a number of known and suspected inhibitors of aldehyde dehydrogenase-catalyzed oxidation of acetaldehyde to acetic acid, e.g., disulfiram and certain cephalosporins (32), that are occasionally administered to cancer patients receiving oxazaphosphorines. It is also known that at least some of these agents inhibit aldehyde dehydrogenase-catalyzed oxidation of 4-hydroxycyclophosphamide/aldophosphamide to carboxyphosphamide as well. Thus, our finding that critical normal cells contain substantial quantities of the relevant aldehyde dehydrogenase activity suggests that inclusion in the therapeutic regimen of agents that inhibit this activity will result in unfavorable drug interactions.

REFERENCES

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