6-Thioguanine-induced Growth Arrest in 6-Mercaptopurine-resistant Human Leukemia Cells

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ABSTRACT

The thiopurines 6-thioguanine (6TG) and 6-mercaptopurine (6MP) are cytotoxic to proliferating cells by a mechanism involving incorporation into DNA, via the purine salvage pathway, and resistance to these agents can be conferred by lack of the salvage pathway enzyme hypoxanthine-guanine phosphoribosyltransferase. However, human and murine hypoxanthine-guanine phosphoribosyltransferase-deficient leukemia cell lines have been shown to respond to 6TG by growth arrest and differentiation by a mechanism apparently not involving incorporation of 6TG into DNA. If so, leukemia cells resistant to 6MP should still respond to 6TG by growth arrest via an undescribed epigenetic mechanism. To test this, polyclonal 6MP-resistant variants were produced from three human leukemia cell lines, HL-60, U937, and CCRF-CEM. Treatment of both sensitive and resistant cells with 6TG induced growth arrest. The effect of 6TG in the 6MP-sensitive HL-60 and U937 cells was associated with significant loss of viability and DNA fragmentation. In contrast, the 6TG-treated 6MP-resistant cells exhibited a slower decline in viability and no DNA fragmentation. To identify the mechanism by which 6TG may induce growth arrest, tRNA was isolated from 6MP-resistant cells cultured for 48 h with 6TG. 6TG was found to be incorporated into tRNAs normally containing queuine in the anticodon wobble position. These studies may provide a basis for the development of new therapeutic regimens for the treatment of leukemia.

INTRODUCTION

Despite much activity in the development and application of chemotherapy agents, new chemotherapeutic compounds and therapeutic strategies are needed that will provide effective treatment of both rapidly and slowly proliferating cancers, while minimizing undesirable side effects. One approach to this problem is to use existing chemotherapeutic agents in novel ways. We have used this approach to identify an apparent epigenetic mechanism of growth control that may provide the basis for a new application of known agents as well as development of new compounds for cancer chemotherapy.

The thiopurines 6TG and 6MP have been part of chemotherapeutic regimens for the treatment of childhood and adult leukemias since the 1950s (1). These agents are cytotoxic to proliferating cells by a mechanism which requires conversion to 6TG nucleotides via the purine salvage pathway, followed by incorporation into DNA in place of the unusual base queuine in the anticodon wobble position. This highly modified purine is found only in the anticodon wobble position of tRNA isoacceptors for asparagine, aspartic acid, histidine, and tyrosine. The modification, which is highly conserved evolutionarily, occurs via a base exchange mechanism catalyzed by the enzyme queuine tRNA-phosphoribosyltransferase (tRNA-GRT; tRNA-guaine:queuine tRNA ribosyltransferase; EC 2.4.2.29). We have shown that treatment of HGPRT-deficient HL-60 cells with 6TG in the presence of additional exogenous queuine (1 µM supplementing the 10–30 nm present in culture medium) prevented the 6TG-induced growth inhibition (7). This evidence implicates an epigenetic mechanism for growth arrest involving the incorporation of 6TG into DNA.

We have proposed that if 6TG causes growth arrest and differentiation in leukemia cell lines by a mechanism not involving HGPRT, then cells that have acquired resistance to 6MP due to lack of salvage pathway activity should nevertheless be sensitive to 6TG-induced growth arrest. To test this, we produced 6MP-resistant variants of three human leukemic cell lines and assessed the effect of 6TG on tRNA modification and cell proliferation. The results could have important implications regarding the development of new therapeutic regimens for leukemia.

MATERIALS AND METHODS

Cell Lines. The promyelocytic leukemia cell line HL-60 was obtained from Dr. Robert Gallo (National Cancer Institute, Bethesda MD); the HGPRT-deficient HL-60 variant was provided by Dr. Linda F. Thompson (Scripps Clinic and Research Foundation, La Jolla CA); U937 cells were provided by Dr. Kathleen Clouse (Georgetown University, Washington DC); and CCRF-CEM cells were obtained from the American Type Culture Collection (Rockville MD). All cells were cultured in RPMI 1640 (GIBCO/BRL, Grand Island, NY) supplemented with 10% FBS (Hyclone Laboratories, Logan, UT) at 37°C in 7% CO2. Cells were subcultured every 5 to 7 days to a density of 2 × 105 cells/ml.

Selection of HGPRT-deficient Cells. Cells were cultured in 25-cm2 tissue culture flasks (Corning) in RPMI 1640 medium supplemented with 10% FBS and increasing concentrations of 6MP (0.4, 0.8, 2, 4, 10, 40, 80, and 350 µM; 1.4, 2, and 3 mM). The concentration of 6MP in the cultures was increased when the growth rate of cells in the presence of 6MP was comparable to that of the parent cells. After several passages at the highest 6MP concentration, salvage pathway activity was assessed as the ability of cells to incorporate [3H]hypoxanthine into cellular nucleic acids.

Received 4/6/94; accepted 8/17/94.

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1 This study was supported by American Cancer Society Grants CH-396 and DHP-37 (to R. W. T. and C. J. M.).

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3 The abbreviations used are: 6TG, 6-thioguanine; 6MP, 6-mercaptopurine; HGPRT, hypoxanthine-guanine phosphoribosyltransferase; FBS, fetal bovine serum; CMF-FBS, calcium, magnesium-free phosphate-buffered saline; TCA, trichloroacetic acid.

The cytotoxic effects of 6MP and 6TG depend on the conversion of the purine bases to nucleotides by the salvage pathway enzyme HGPRT; absence of this enzyme is the best characterized mechanism of thiopurine resistance (reviewed in Ref. 3). Nevertheless, Friend erythroleukemia cells lacking HGPRT respond to treatment with 6TG in vitro by growth arrest and cellular differentiation, in the absence of incorporation of 6TG nucleotides into DNA (4). A similar effect of 6TG in HGPRT-deficient HL-60 human acute promyelocytic leukemia cells has been shown to be induced by the free base 6TG rather than other metabolites (5). We have demonstrated previously that 6TG becomes incorporated into the anticodon of specific tRNA isoacceptors of HGPRT-deficient HL-60 cells in place of the unusual base queuine (6). This highly modified purine is found only in the anticodon wobble position of tRNA isoacceptors for asparagine, aspartic acid, histidine, and tyrosine. The modification, which is highly conserved evolutionarily, occurs via a base exchange mechanism catalyzed by the enzyme queuine tRNA-phosphoribosyltransferase (tRNA-GRT; tRNA-guaine:queuine tRNA ribosyltransferase; EC 2.4.2.29). We have shown that treatment of HGPRT-deficient HL-60 cells with 6TG in the presence of additional exogenous queuine (1 µM supplementing the 10–30 nM present in culture medium) prevented the 6TG-induced growth inhibition (7). This evidence implicates an epigenetic mechanism for growth arrest involving the incorporation of 6TG into DNA.

We have proposed that if 6TG causes growth arrest and differentiation in leukemia cell lines by a mechanism not involving HGPRT, then cells that have acquired resistance to 6MP due to lack of salvage pathway activity should nevertheless be sensitive to 6TG-induced growth arrest. To test this, we produced 6MP-resistant variants of three human leukemic cell lines and assessed the effect of 6TG on tRNA modification and cell proliferation. The results could have important implications regarding the development of new therapeutic regimens for leukemia.

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**RESULTS**

Derivation of Purine Salvage-deficient Cell Lines. HGPRT-deficient HL-60 cells are growth inhibited and induced to differentiate by 6TG (4, 6, 7). 6TG and 6MP are thought to use a single pathway, requiring this enzyme, to cause cytotoxicity. We wished to determine specifically whether cells resistant to 6MP retained responsiveness to 6TG. Three human leukemia cell lines were used in these studies: HL-60, derived from a patient with acute promyelocytic leukemia (12); CCRF-CEM, a T-lymphoblast line derived from a patient with acute lymphoblastic leukemia (13); and U937, a monocyte-like cell line derived from a patient with histiocytic lymphoma (14). 6MP-resistant variants of these cell lines were produced by culture in RPMI 1640 containing 10% FBS and increasing concentrations of 6MP (Fig. 1). After several passages in 6MP, resistant cells were cultured in the absence of 6MP. To approximate the in vivo acquisition of resistance to therapeutic 6MP, which may be polyclonal in origin, we did not attempt to isolate clonal cell lines from these cultures.

Purine salvage capability was assessed by measuring the incorporation of [3H]hypoxanthine into TCA-precipitable material (Table 1). Incorporation of [3H]hypoxanthine in 6MP-deficient HL-60 (HL-60)<sup>Δ</sup> cells was less than 1% of that in the 6MP-sensitive HL-60 cells. This value was very similar to that for incorporation of [3H]hypoxanthine into nucleic acid by an independently obtained HGPRT-deficient HL-60 cell line (HL-60<sup>Δ</sup>). Similarly, HGPRT activity in U937<sup>Δ</sup> cells was less than 1% of the parent U937 cell line. In contrast, 6MP-resistant CCRF-CEM (CEM<sup>Δ</sup>) cells retained a significant fraction

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**6TG ARREST IN 6MP-RESISTANT LEUKEMIA CELLS**

[Image and text content as per the original document]
Fig. 2. Growth (left panels) and viability (right panels) of 6MP-resistant cell lines in the absence or presence of 6TG. Cells were subcultured from late log phase to a density of $2 \times 10^5$ cells/ml into RPMI 1640–10% FBS in the absence or presence of 0.4 mM 6TG. Cell number was assessed by counting aliquots of each culture daily using a hemacytometer. Viability was evaluated using 0.2% trypan blue. These curves are representative of many repeated experiments. A and E, HL-60 $^+$; B and F, HL-60 $^-$; C and G, U-937 $^+$; D and H, CEM $^+$. 

6TG ARREST IN 6MP-RESISTANT LEUKEMIA CELLS
**Table 1** Incorporation of \[^{3}H\]\hypoxanthine

<table>
<thead>
<tr>
<th>Cell line</th>
<th>cpm</th>
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</thead>
<tbody>
<tr>
<td>HL-60</td>
<td>15085</td>
</tr>
<tr>
<td>HL-60(r)</td>
<td>116</td>
</tr>
<tr>
<td>CEM(r)</td>
<td>46846</td>
</tr>
<tr>
<td>CEM(d)</td>
<td>3632</td>
</tr>
<tr>
<td>U-937d</td>
<td>49466</td>
</tr>
<tr>
<td>U-937(d)</td>
<td>417</td>
</tr>
</tbody>
</table>

\(^{a}\) Cells (2 × 10\(^{5}\)) were incubated for 1 hour with 1 μCi \[^{3}H\]\hypoxanthine in RPM 1640-10% FBS. Cells were disrupted, and nucleic acids were precipitated with 10% TCA as described in "Materials and Methods." Shown are cpm per 2 × 10\(^{5}\) cells from one representative experiment.

(8%) of the HGPRT activity of the parent cell line. The salvage-deficient phenotypes are stable over many passages in 6MP-free medium (data not shown); currently, cells are passaged in 1 mM 6MP at 4-week intervals. However, despite continued culture in 6MP, incorporation of \[^{3}H\]\hypoxanthine into nucleic acids by CEM\(d\) cells has not declined to the level of HL-60\(r\) or U937\(d\).

**Effect of 6TG on Proliferation and Viability of 6MP-sensitive and Resistant Cells.** To determine if 6TG could inhibit the growth of leukemia cells resistant to 6MP, cells were subcultured from late logarithmic growth into medium containing or lacking 0.4 mM 6TG (Fig. 2). In the presence of 6TG, the growth of HL-60\(r\) (Fig. 2B), U937\(d\) (Fig. 2C), and CEM\(d\) (Fig. 2D) cells was arrested within 72 to 96 h of treatment. The proliferation of HL-60\(d\) cells was affected similarly to that of the HGPRT-deficient cell line HL-60\(r\) (Fig. 2A). In contrast, 6MP-resistant cells cultured in medium alone proliferated normally. The viability of 6MP-resistant cells cultured in 6TG remained high (>60%) for at least 3 days, declining gradually thereafter in the HL-60\(d\) (Fig. 2F) and CEM\(d\) (Fig. 2H) cells but remaining at 60 to 70% through day 7 in the U937\(d\) cells (Fig. 2G). In numerous experiments, day 3 viabilities of these cells in 6TG remained high, and

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**Fig. 3.** Growth (left panels) and viability (right panels) of parent cell lines in the absence or presence of 6TG. Cells were cultured and evaluated as described in Fig. 2. A and D, HL-60; B and E, U-937; C and F, CEM.
day 5 viabilities varied from 10 to 80%. In the absence of 6TG, 6MP-resistant cells remained greater than 90% viable until saturation densities were reached (days 5–6).

To compare the effect of 6TG on 6MP-resistant cells with that on cells with an intact purine salvage pathway, the parent cell lines were cultured in parallel in the presence and absence of 0.4 mM 6TG (Fig. 3). The proliferation of the parent cell lines in the absence of 6TG was very similar to that of the untreated 6MP-resistant variants. However, in the presence of 6TG, little accumulation and extensive cell death was observed. The decline of cell viability of HL-60 and U937 cells with an intact purine salvage pathway, the parent cell lines were densities were reached (days 5—6).

6MP-resistant cells remained greater than 90% viable until saturation day 5 viabilities varied from 10 to 80%. In the absence of 6TG, tRNA was fractionated by reverse-phase chromatography on an RPC-5 column, and column fractions were dot blotted onto nylon membranes. tRNA isoacceptors for histidine, tyrosine, asparagine, and aspartic acid were identified by consecutive hybridization of each blot with antisense oligonucleotides derived from the 3’ ends of known human and murine tRNA sequences (11). Elution profiles of tRNA isoacceptors for histidine and tyrosine generated by laser densitometry of the resulting Autoradiographs are shown in Fig. 5. A shift of the tRNA profiles of treated cells to higher salt concentrations for all four tRNA isoaccepting species indicates the incorporation of 6TG into tRNA (11). The extent of 6TG incorporation into tRNAs depended upon the cell line and tRNA analyzed. Incorporation of 6TG into tRNA isoacceptors for histidine, tyrosine, and asparagine (data not shown) was observed in all three 6MP-resistant cell lines and was most evident in CEMd cells. In contrast, less incorporation of 6TG into aspartate isoacceptors was observed in the HL-60d, CEMd, or U937d cells (data not shown).

**DISCUSSION**

The chemotherapeutic agents 6MP and 6TG have been presumed to cause cytotoxicity by a common mechanism involving incorporation of 6TG nucleotides into DNA via the purine salvage pathway. However, the ability of 6TG to inhibit the growth of HOPRT-deficient Friend erythroleukemia cells without incorporation into DNA suggests that an alternative, epigenetic mechanism of 6TG-induced arrest exists that may be operational in cells lacking purine salvage capability. If so, then cells that had acquired resistance to 6MP, often via loss of purine salvage activity, should retain sensitivity to 6TG. To test this, we determined the effect of 6TG on proliferation in 6MP-resistant leukemia cells of the myeloid, monocytic, and lymphoid lineages. In each lineage, resistance to 6MP was associated with loss or diminution of purine salvage capability, indicated by inability to incorporate [3H]hypoxanthine into DNA. Treatment of 6MP-resistant cells with 6TG resulted in growth inhibition, which differed from the toxic effect of 6TG in the parental, purine salvage competent cell lines in several ways: (a) while 6TG treatment induced growth arrest within 24 h in the parent cells, the kinetics of arrest was delayed in the resistant cells, becoming apparent only after 2 to 4 days of culture; and (b) growth inhibition in the HL-60 and U937 cell lines was associated with a precipitous loss of cell viability, whereas viability remained high for as much as a week in the 6MP-resistant cells treated with 6TG. 6TG arrest in the purine salvage-competent HL-60 cell line was associated with DNA fragmentation. Treatment of salvage-competent U937 cells for 24 h also resulted in DNA fragmentation. The nature of the gradual loss of viability of the CCRF-CEM and 6MP-resistant cells is less clear. DNA fragmentation was not readily identifiable in the salvage-competent CCRF-CEM cells or in the 6MP-resistant HL-60, U937, and CEM cells treated for 3 days with 6TG, despite...
viabilities as low as 66%. We also assessed the extent of apoptotic cell death in treated and untreated cultures stained with acridine orange; the results were consistent with those obtained by analysis of DNA fragmentation (data not shown). Studies are in progress to clarify the role of programmed cell death in the loss of viability observed in 6MP-resistant cell lines treated with 6TG.

The concentration of 6TG used in these studies, 0.4 mM, is higher than required for killing of purine salvage-competent cells in vitro but considerably less than the highest concentration of 6MP to which these cells are known to be resistant (2 mM). It is difficult to relate these figures to therapeutic 6TG levels. Oral or i.v. 6TG is commonly administered at a dose of 55 to 100 mg/m² (0.5 to 1 mmol in an average size patient) every 12 h (15, 16). Peak plasma thioguanine levels are highly variable, in the range of 0.03 to 0.94 μM in one study (16). In children receiving comparable levels of 6MP, peak erythrocyte accumulation of 6TG nucleotides is highly variable, ranging from 100 to 600 pmol per 8 × 10⁸ cells (17). It is unclear how these values reflect concentrations of 6TG at cellular targets. Thus, it is difficult to compare the concentration of 6TG used here with actual therapeutic levels. We note, however, that the concentration of 6TG used in these experiments is similar to the concentration of 6MP required to inhibit 60% mixed lymphocyte responses in cells obtained from HGPRT-deficient Lesch-Nyhan patients (18).

Both CEM and CEM⁶ cells responded to treatment with 6MP and 6TG differently from the myeloid and monocytic leukemia cells. CEM⁶ cells resistant to high concentrations of 6MP retained significant purine salvage capability. This may reflect the greater use of the de novo pathway of purine synthesis, rather than purine salvage, thought to be characteristic of T lymphocytes.

We demonstrated previously that 6TG became incorporated into tRNA isoacceptors of the queuine-containing family in HGPRT-deficient HL-60 cells and that 6TG-induced growth inhibition in these cells could be prevented by the addition of exogenous queuine (6, 7). Since queuine has been found only in the wobble position of specific tRNAs (19), 6TG-induced growth inhibition in these cells may occur via a tRNA-mediated mechanism. Analysis of tRNA isoacceptors for histidine and aspartic acid indicates that 6TG is variably incorporated into these tRNAs in the 6MP-resistant cells within 48 h. The extent of incorporation of 6TG in these cells should depend on several factors. tRNA isoacceptors for aspartate are the preferred substrate for tRNA-GRT (20) and thus are expected to be relatively more queuine-modified than the other queuine-containing tRNAs. Since queuine incorporation into tRNA is irreversible, only unmodified and newly synthesized tRNAs would be expected to be substrates for 6TG incorporation. Therefore, preexisting tRNA isoacceptors for aspartic acid would be less sensitive to 6TG modification, and histidine tRNA isoacceptors, which are poorer substrates for tRNA-GRT, would be expected to incorporate 6TG relatively well. Among newly synthesized tRNAs, aspartic acid isoacceptors would be expected to be preferentially 6TG modified. However, since the incorporation of 6TG into tRNA is a reversible process, incorporated 6TG should ultimately be replaced by queuine from the serum. Such transient 6TG modification has been observed in HL-60⁶ cells (6). The reason for the greater 6TG modification in CEM⁶ cells, compared to HL-60⁶ and
U937\textsuperscript{d} cells, is under investigation. CEM cells may be queuine hypomodified to a greater degree (see below) or may be more actively synthesizing new tRNAs.

Treatment of the 6MP-resistant cells with 6TG in the presence of exogenous queuine prevented the 6TG-induced growth arrest (Table 2), suggesting that in 6MP-resistant cells, 6TG-induced growth arrest may occur by a tRNA-mediated mechanism. In view of the relatively high viability of the CCRF-CEM cells early after arrest in 6TG, we speculate that early arrest in these cells may also involve incorporation of 6TG into tRNA.

The role of queuine modification in tRNA is still unclear. Abundant evidence suggests that queuine modification is important for normal cell function (reviewed in Ref. 20). tRNAs in normal tissues tend to be completely queuine-modified, despite the fact that eukaryotes must obtain queuine from the diet and gut flora. tRNAs of numerous human and experimental tumors have been shown to be hypomodified for queuine, and introduction of excess exogenous queuine can cause tumor regression in animal models. In human leukemias and lymphomas, there is a strong correlation between the extent of hypomodification and prognostic stage (21). tRNA isolated from a T-lymphoblastic lymphoma was found to be highly undermodified, consistent with our observations of the relatively efficient incorporation of 6TG into the CEM\textsuperscript{d} cells. Interestingly, in patients with such B-cell malignancies as CLL stage A and hairy cell leukemias, queuine hypomodification was not restricted to rapidly proliferating leukemias, suggesting that it is not generally a reflection of cell proliferation. In vitro, numerous transformed cell lines are queuine hypomodified (20, 22), and treatment of methylcholanthrene-initiated primary hamster fibroblasts with 7-methylguanine, which inhibits tRNA-GRT, generated cells exhibiting anchorage-independent growth and queuine hypomodification (23).

The molecular consequences of 6TG incorporation into tRNA are unknown. tRNA\textsuperscript{Tyr} modified with 6-thiouridine can act as an amber suppressor, suggesting that queuine modification may be important for translation fidelity (24). Queuine-containing tRNAs exhibit less preferential codon recognition than G-containing tRNAs (25), and specific proteins are not expressed in queuine-deficient \textit{D. discoideum} and \textit{Escherichia coli}, suggesting that queuine modification may play a role in the posttranscriptional regulation of protein expression (26, 27). Such a regulatory mechanism would be most important for the regulation of synthesis of proteins that are rapidly synthesized and degraded, such as those involved in cellular signaling. Interference with the appropriate expression of these proteins could cause the growth arrest and perhaps the gradual cell death observed here. In addition, we and others have observed induction of differentiation in HGRT-deficient leukemia cell lines treated with 6TG, an effect which could also be triggered by alterations in the appropriate synthesis of regulatory proteins.

These data suggest that growth arrest can be induced in leukemia cells by an epigenetic mechanism, possibly involving alterations in tRNA queuine modification. Although the use of 6TG for this purpose in purine salvage-competent individuals would be complicated by the incorporation of 6TG into DNA, 6TG may be an effective therapeutic strategy in patients that have acquired resistance to 6MP. Moreover, the identification of purine analogues capable of acting as substrates for tRNA-GRT but not HGRT may provide a new class of chemotherapeutic agents which are not DNA synthesis-directed. The effectiveness of such agents may not be restricted to rapidly proliferating tumors but may also be effective in the treatment of slowly proliferating, queuine hypomodified leukemias.

ACKNOWLEDGMENTS

We thank Marsha Stalker and Carol LeMay for their secretarial assistance with this manuscript.

REFERENCES

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