Differing Contribution of Thiopurine Methyltransferase to Mercaptopurine versus Thioguanine Effects in Human Leukemic Cells

Thierry Dervieux, Javier G. Blanco, Eugene Y. Krynetski, Elio F. Vanin, Martine F. Roussel, and Mary V. Relling


ABSTRACT

Thioguanine and mercaptopurine are prodrugs requiring conversion into thiopurine nucleotides to exert cytotoxicity. Thiopurine S-methyltransferase (TPMT), an enzyme subject to genetic polymorphism, catalyzes thiopurines into inactive methylated bases, but also produces methylthioguanine nucleotides and methylmercaptopurine nucleotides from thioguanine and mercaptopurine nucleotides, respectively. To study the effect of TPMT on activation versus inactivation of mercaptopurine and thioguanine, we used a retroviral gene transfer technique to develop human CCRF-CEM cell lines that did (TPMT⁺) and did not (MOCK) overexpress TPMT. After transduction, TPMT activities were 14-fold higher in the TPMT⁺ versus the MOCK cell lines (P < 0.001). TPMT⁺ cells were more sensitive to thioguanine than MOCK cells (IC₅₀ = 1.10 ± 0.12 μM versus 0.55 ± 0.19 μM; P = 0.02); in contrast, TPMT⁺ cells were more sensitive to mercaptopurine than MOCK cells (IC₅₀ = 0.52 ± 0.20 μM versus 1.50 ± 0.23 μM; P < 0.01). The lower sensitivity of TPMT⁺ versus MOCK cells to thioguanine was associated with low thioguanine nucleotide concentrations (917 ± 282 versus 1515 ± 183 pmol/5 × 10⁶ cells; P = 0.01), higher methylthioguanine nucleotide concentrations (252 ± 34 versus 27 ± 10 pmol/5 × 10⁶ cells; P = 0.01), less inhibition of de novo purine synthesis (13 versus 95%; P < 0.01), and lower deoxothioguanosine incorporation into DNA (2.0 ± 0.6% versus 7.2 ± 2.0%; P < 0.001). The higher sensitivity of TPMT⁺ cells to mercaptopurine was associated with higher concentrations of methylmercaptopurine nucleotide (2601 ± 1055 versus 174 ± 77 pmol/5 × 10⁶ cells; P = 0.01) and greater inhibition of de novo purine synthesis (>99% versus 74%; P < 0.01) compared with MOCK cells. We conclude that methylation of mercaptopurine contributes to the antiproliferative properties of the drug, probably through inhibition of de novo purine synthesis by methylmercaptopurine nucleotides, whereas thioguanine is inactivated primarily by TPMT.

INTRODUCTION

Mercaptopurine and thioguanine are thiopurine drugs currently administered in the treatment of acute lymphoblastic and myeloblastic leukemia. Their precise mechanism of action remains unclear despite >40 years of in vivo and in vitro investigations (1). Mercaptopurine and thioguanine require intracellular conversion by HPRT³ into thiopurine nucleotides to exert cytotoxicity (2). In cells, mercaptopurine (the analogue of hypoxanthine) and thioguanine (the analogue of guanine) are converted by HPRT into TMP and TGNP, respectively, and subsequently to the active cytotoxic TGNs, which are incorporated into DNA in the reduced form (deoxythioguanosine; Refs. 3–5). TGNP and TMPG are methylated by TPMT (EC 2.1.1.67), an S-adenosylmethionine-dependent cytosolic enzyme, into MeTGNP and MeTMPG, respectively (Fig. 1; Refs. 6, 7).

TPMT is subject to a common genetic polymorphism, with ~10% of individuals homozygous and 0.3% homozygous mutant at the TPMT locus (8). Patients with low TPMT activity have greater hematological toxicity after standard doses of mercaptopurine, thioguanine, or azathioprine than do patients with wild-type TPMT (9–12). Such patients have very high concentrations of active TGNs, with low or absent methylthiopurine metabolite concentrations. However, the potential contributions of MeTGNP and MeTPMT to the pharmacological properties of thioguanine and mercaptopurine, respectively, remain unclear. Methylation of mercaptopurine and thioguanine bases is an inactivating pathway, shunting parent drugs away from the HPRT activation pathway. However, methylation of TGNP or administration of methylmercaptopurine riboside results in inhibition of DNPS (13, 14). In vivo observations show that patients with low TPMT activity tolerate higher concentrations of TGNs than do patients with wild-type TPMT (9, 10, 15). In addition, incorporation of deoxothioguanosine into DNA of various human B-lineage and T-lineage cell lines does not correlate with their sensitivity to mercaptopurine (5). Thus, these in vivo and in vitro data suggest that methylation could contribute to the effects of thiopurines.

To clarify whether and by what mechanism TPMT contributes to cytotoxicity, we have developed isogenic human leukemic cell lines expressing recombinant human TPMT and compared the cytotoxic effects of mercaptopurine and thioguanine.

MATERIALS AND METHODS

Cell Lines and Culture Conditions. Human kidney 293T cells, murine fibroblast NIH-3T3 cells, and human HeLa cells were maintained in DMEM (BioWhittaker, Walkersville, MD) supplemented with 10% heat-inactivated fetal bovine serum (Hyclone, Logan, UT) and 2 mM glutamine. Human CCRF-CEM cells (human acute lymphoblastic leukemia, T lineage) were purchased from the American Type Culture Collection and were maintained in RPMI 1640 (BioWhittaker) supplemented with 10% heat-inactivated fetal bovine serum and 2 mM glutamine. All cells were maintained in log phase at 37°C in a 5% CO₂, humidified atmosphere.

Preparation of Replicative Incompetent Retroviruses. The functional human TPMT cDNA (16) was subcloned into the MSCV-IREG-GFP vector at the EcoRI and NotI sites upstream of an IRES, driving the expression of GFP (17). All plasmids were purified by standard cesium chloride gradient centrifugation. Retroviruses were prepared by transient cotransfection of the vector and helper plasmids in the packaging cell line 293T cells (18). Murine amphotropic retroviruses were prepared by cotransfection of 15 μg of MSCV-IREG-GFP (MOCK vector) or MSCV-TPMT-IREG-GFP (TPMT + vector) and 15 μg of the helper plasmid PEQ-PAM3 (encoding the envelope, nucleocapside, and reverse transcriptase) by standard calcium phosphate precipitation (19). RD114-pseudotyped retroviruses containing the feline endogenous virus envelope (20) were cotransfected with 10 μg of MSCV-IREG-GFP or MSCV-TPMT-IREG-GFP vector with 10 μg of helper pRDF (encoding the envelope) and pEQ-PAM3-E (encoding the nucleocapside and reverse transcriptase), using Fugene-6 transfection reagent (Roche Diagnostics, Indianapolis, IN). Twenty-four h after transfection, retroviral supernatants were...
harvested every 6–12 h for up to 72 h. Retroviral supernatants were pooled, filtered, and stored at −70°C.

Titters (number of viral particles per ml of supernatant) were estimated using a 1:10 dilution of the retroviral supernatant. The functional integrity of the RD114 retroviruses was assessed in HeLa cells prior to transduction in CEM.

**Retroviral Transduction of Cells.** Murine NIH-3T3 cells (2.0 × 10^6 cells/10-cm dish) were transduced with amphotropic retroviral supernatants in the presence of 10 μg/ml Polybrene (Sigma Chemical Co., St. Louis, MO). GFP expression was scored by FACS 72 h after transduction. The CEM cells were transduced using the RD114 retroviruses in the presence of Polybrene (final concentration, 8 μg/ml) and retinectin (recombinant human fibronectin fragment CH-296; TAKARA, Otsu, Japan). Retinectin was coated on 6-well Costar plates (Corning Inc., New York, NY) at a concentration of 20 μg/cm², and 4 ml of retroviral supernatant were preloaded on the retinectin over 30 min at 37°C and then discarded. Subsequently, a total of 10³ cells resuspended in 5 ml of retroviral supernatant were loaded on the plates. This transduction procedure was repeated twice over 48 h. CEM cells expressing GFP were counted as the ratio of viable cells incubated with drugs (i.e., cell count times drug concentration) to the number of cells treated with solvent alone. Cell viability was determined by annexin staining. Approximately 5 × 10⁶ cells after incubation of CEM cells with thioguanine or mercaptopurine, using the Qiagen cell culture DNA kit (Qiagen, Chatsworth, CA), according to the manufacturer’s instructions. Quantitation of deoxythioguanosine in genomic DNA was monitored by high-performance liquid chromatography after enzymatic hydrolysis of 10 μg of DNA as reported previously (5).

**Flow Cytometry Analysis and Cell Viability Assessment.** GFP-expressing cells were selected by flow cytometry with a FACS Calibur Flow Cytometer (Becton Dickinson, San Jose, CA) using excitation at 488 nm and fluorescence detection at 530 nm. Propidium iodide (Sigma) was added to all samples before analysis to allow differentiation of viable cells from dead cells. Cell viability was determined by annexin staining. Approximately 5 × 10⁶ cells were washed in PBS and resuspended in 100 μl of staining buffer (10 mM HEPES, 0.9% NaCl, 2.5 mM CaCl₂, 0.1% BSA) containing 10 μl of phycoerythrin-conjugated Annexin-V (Annexin-V-PE; PharMingen, San Diego, CA) and incubated at room temperature in the dark for 15 min, followed by the addition of 500 μl of staining buffer containing 2.5 μg of propidium iodide. The samples were filtered through 40 μm nylon mesh and analyzed on a Becton Dickinson FACS Calibur flow cytometer, collecting green fluorescence from GFP, orange fluorescence from Annexin-V, and red fluorescence from propidium iodide.

**RESULTS**

**Retroviral Gene Transfer and Expression of Human TPMT.** Retroviral titers for the MOCK and the TPMT transductions were transduced in the NIH-3T3 cells with an efficiency ranging from 96 to 100% (percentage of cells gated GFP positive by comparison with a negative control of untransduced cells). Transduction of the TPMT-containing retrovirus in 3T3 cells yielded a 22-fold increase of TPMT activity relative to MOCK cells (P < 0.001), whereas transduction of the retrovirus control (MOCK) had no effects on TPMT activity (P = 0.40 compared with untransduced cells; Fig. 2).
The efficiency of transduction with amphotropic retroviruses was lower with human CCRF-CEM human T lymphoblasts (only 10% of GFP-positive cells for TPMT retrovirus) than with 3T3 cells. To achieve a higher efficiency of transduction, we used a combination of retargeting and RD114-pseudotyped retroviral particles, which allowed adequate recoveries of GFP-positive cells (85 and 40% for the MOCK and TPMT+ retroviruses, respectively; average of two separate experiments). After flow cytometry sorting, CEM-TPMT+ cells had 14-fold higher TPMT activity than did the CEM-MOCK cells (Fig. 2).

Effects of Thioguanine, Mercaptopurine, and Methylmercaptopurine Riboside. Prior to cytotoxicity studies, we determined whether transduction with TPMT could have modified critical parameters of thiourine responsiveness. CEM-TPMT+ cells were comparable to the CEM-MOCK cells with regard to the doubling time (24.4 ± 1.1 versus 24.5 ± 2.1 h; P = 0.82), DNPS rate (23.3 ± 7.8 versus 26.2 ± 8.5 pmol/mmol/h; P = 0.44), and HPRT activities (8.6 ± 5.2 versus 8.6 ± 3.9 pmol/h/10^6 cells; P = 1.0). Similarly, no significant differences in these parameters were found between the 3T3-MOCK and 3T3-TPMT+ cells (not shown).

We investigated the inhibitory effects of mercaptopurine, thioguanine, and methylmercaptopurine riboside on proliferation in the CEM-MOCK and the CEM-TPMT+ cells (Table 1). As expected, after 48 h of incubation with methylmercaptopurine riboside, which bypasses the methylation by TPMT, we observed no differences in sensitivity between the MOCK and TPMT+ cells (P = 0.38). In addition, methotrexate yielded no significant difference of sensitivity (P = 0.27) between MOCK and TPMT+ cells. However, the treatment of cells with mercaptopurine or thioguanine showed significant and opposite differences. After 48 h of incubation with thioguanine, a 2-fold lower IC_{50} for the MOCK cells was observed compared with the TPMT+ cells (P = 0.02); in contrast, after mercaptopurine incubation, a 2.9-fold higher IC_{50} was observed for the MOCK cells compared with the TPMT+ cells (P < 0.01). The TPMT+ cells exhibited a 2-fold lower IC_{50} after mercaptopurine treatment compared with after thioguanine treatment (P = 0.01). In contrast, MOCK cells had a 2.7-fold higher IC_{50} after mercaptopurine treatment compared with after thioguanine treatment (P = 0.01). A similar pattern of sensitivity was seen in the 3T3 recombinant cells: after 48 h of incubation with mercaptopurine (10 μM), we observed a greater inhibition of proliferation (P = 0.01) in the 3T3-TPMT+ cells (inhibition 73 ± 14% relative to control) than in the 3T3-MOCK cells (inhibition 47 ± 10% relative to control).

We determined cell viability by annexin staining after 24 or 48 h of treatment with mercaptopurine (10 μM), thioguanine (1 μM), or methylmercaptopurine riboside (1 μM) in the CEM recombinant cells. For all compounds tested, no significant cytotoxic effect was observed after 24 h of treatment (viability >90% compared with untreated controls); this is consistent with the delayed effects of thiopurines. After 48 h of incubation with mercaptopurine, lower viability was observed in the CEM-TPMT+ cells compared with CEM-MOCK cells (P < 0.01; Fig. 3). In contrast, after thioguanine treatment, higher viability was observed in the CEM-TPMT+ cells compared with the CEM-MOCK cells (P < 0.01). No significant differences in cell viability were observed after 48 h of incubation with methylmercaptopurine riboside in the MOCK cells compared with TPMT+ cells (P = 0.54).

Cytosolic Thiopurine Metabolites. We compared the cytosolic thiopurine nucleotide concentrations in the MOCK and TPMT+ cells after incubation with mercaptopurine (10 μM), thioguanine (1 μM), and methylmercaptopurine riboside (1 μM). After 24 h incubation with mercaptopurine, the CEM-TPMT+ cells had lower concentrations of TINS (P = 0.007), comparable levels of TGNs (P = 0.8), and 15-fold higher concentrations of MeMPNs (P < 0.001) than the CEM-MOCK cells (Fig. 4). The total thiopurine nucleotide concentration (TIN + TGN + MeMPN) in the CEM-MOCK cells (7926 ± 2143 pmol/5 × 10^6 cells) was similar to that of the CEM-TPMT+ cells (7621 ± 3590 pmol/5 × 10^6 cells; P = 0.72). After incubation for 48 h with mercaptopurine, TGN levels were lower in the CEM-TPMT+ cells (100 ± 92 pmol/5 × 10^6 cells) than in the CEM-MOCK cells (298 ± 49 pmol/5 × 10^6 cells; P < 0.05). Similarly, after 48 h of incubation with mercaptopurine (10 μM), 3T3-TPMT+ cells had lower concentrations of TGN compared with ...

Table 1 Average IC_{50} (± SD) for thioguanine, mercaptopurine, methylmercaptopurine riboside, and methotrexate in CEM-MOCK and CEM-TPMT+ cells after 48 h of incubation

<table>
<thead>
<tr>
<th>Compound</th>
<th>MOCK</th>
<th>TPMT+</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thioguanine</td>
<td>0.55 ± 0.19</td>
<td>1.10 ± 0.12</td>
<td>0.02</td>
</tr>
<tr>
<td>Mercaptopurine</td>
<td>1.50 ± 0.23</td>
<td>0.52 ± 0.20</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Methylmercaptopurine</td>
<td>0.072 ± 0.046</td>
<td>0.054 ± 0.029</td>
<td>0.38</td>
</tr>
<tr>
<td>Riboside</td>
<td>0.012 ± 0.005</td>
<td>0.012 ± 0.006</td>
<td>0.27</td>
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Inhibition of proliferation was determined using the CellTiter 96 AQueous Assay (Promega) with colorimetric detection of formazan formed at 490 nm. Results summarize three to five experiments at different passage numbers, with four replicates each.
3T3-MOCK cells (67 ± 46 pmol/5 × 10⁶ cells versus 124 ± 58 pmol/5 × 10⁶ cells; P = 0.05) and higher concentrations of MeMPN (7296 ± 4385 pmol/5 × 10⁶ cells versus 655 ± 207 pmol/5 × 10⁶ cells; P < 0.01; not shown).

Thioguanine, which is directly processed by HPRT into TGNs, also showed significant differences between CEM-MOCK and CEM-TPMT+ cells. After 24 h of incubation with thioguanine (1 μM), we observed significantly lower TGN concentrations in the TPMT+ compared with the MOCK cells (P < 0.01), and 9-fold higher concentrations of MeTGNs (P < 0.01) in TPMT+ cells (Fig. 4). However, the total concentration of thiopurine nucleotides after thioguanine incubation was slightly but significantly (P = 0.02) higher in the MOCK (1542 ± 192 pmol/5 × 10⁶ cells) compared with the TPMT+ cells (1132 ± 215 pmol/5 × 10⁶ cells). After 24 h of treatment with methylmercaptopurine riboside, comparable MeMPN levels in the MOCK and the TPMT+ cells were observed (4383 ± 475 versus 4471 ± 599 pmol/5 × 10⁶ cells; P = 0.58). Thus, the higher cytotoxic effect observed in the TPMT+ cells compared with MOCK cells after mercaptopurine incubation was correlated with higher MeMPN levels, whereas the higher survival of the TPMT+ cells compared with the MOCK cells after thioguanine incubation was correlated with lower TGN concentrations.

**Incorporation of Deoxythioguanosine into Genomic DNA.** The percentage of incorporation of deoxythioguanosine into genomic DNA, expressed as the percentage of all guanosine substituted by deoxythioguanosine after mercaptopurine (10 μM) or thioguanine (1 μM) treatment, is shown in Fig. 5. After 24 or 48 h of treatment with mercaptopurine, CEM-MOCK cells had 1.6-fold higher percentage of deoxythioguanosine incorporation into their DNA compared with CEM-TPMT+ cells (P < 0.01). After 24 h of treatment with thioguanine, CEM-MOCK cells had 1.7-fold higher percentage of deoxythioguanosine incorporation (P < 0.01), which reached a 3.6-fold higher range at 48 h (P < 0.01) when compared with the CEM-TPMT+ cells. Thus, after 48 h of treatment, the percentage of incorporation of deoxythioguanosine into the DNA of MOCK cells treated with thioguanine was higher than that observed after MOCK cells were treated with mercaptopurine (P = 0.03).

**Effects on DNPS.** The CEM-TPMT+ cells treated with mercaptopurine (10 μM) exhibited more rapid and pronounced inhibition of DNPS compared with CEM-MOCK cells (Fig. 6). The DNPS rate was inhibited by >95% in the CEM-TPMT+ cells after only 12 h of treatment, whereas complete inhibition was observed in the CEM-MOCK cells only after 48 h. In contrast, CEM-TPMT+ cells treated with thioguanine (1 μM) had only 13% DNPS inhibition after 48 h of treatment, whereas the CEM-MOCK cells showed 95% inhibition (P < 0.01). After 48 h of treatment with mercaptopurine (10 μM), DNPS was completely inhibited in the 3T3-TPMT+ cells (99.6 ± 0.3%), whereas in the 3T3-MOCK cells, we observed a lower level of inhibition (65.2 ± 7.7%; P = 0.02).

The MeMPN concentration after mercaptopurine treatment in the CEM-MOCK and TPMT+ cells was highly correlated with the percentage of DNPS inhibition (R = −0.94; P < 0.001), and the concentration of MeMPNs inducing a 50% inhibition of DNPS was 224 pmol/5 × 10⁶ cells (Fig. 7). TGN concentrations did not correlate with DNPS inhibition after mercaptopurine treatment (P > 0.90), but in the case of thioguanine treatment, higher TGN concentrations correlated with higher DNPS inhibition in the CEM recombinant cells (P = 0.02; data not shown).

**DISCUSSION**

TPMT, a cytosolic enzyme subject to a common genetic polymorphism, is partly responsible for variations in thiopurine response among patients. Patients with low TPMT activities are at greater risk of hematological side effects during thiopurine treatment and exhibit increased intracellular accumulation of active nonmethylated TGNs (9, 10). Methylation of mercaptopurine and thioguanine bases is inactivating, leaving less parent substrate available for activation through the HPRT pathway. The mechanism of antitumor action of thiopurines is hypothetically related to the incorporation of TGNs into DNA (2, 4). This idea is supported by the fact that the inhibition of this incorporation protects cells against the delayed effect of mercaptopurine or thioguanine (4, 26). However, there are several pieces of evidence that support the role of TPMT in the regulation of thiopurine metabolism and response.
We chose CCRF-CEM cells because they are known to be sensitive to thiopurines and they express relatively low TPMT activity constitutively (7). The 14-fold higher TPMT activity we observed in our TPMT+ cells compared with the MOCK cells after transduction shows a similar fold range of activity (lowest to highest) as has been observed in leukemic blasts of patients with acute lymphoblastic leukemia (29-fold; Ref. 23). In addition, we found comparable effects of TPMT on thiopurine pharmacology in murine NIH-3T3 cells and in human CEM cells. We acknowledge that the absolute level of TPMT in both the MOCK and TPMT+ CCRF-CEM cells is higher than the levels measured in patient leukemic blasts (3.3–88.9 units/10^9 cells; Ref. 23), and thus caution should be exercised in extrapolating the results presented here directly to the clinical situation. However, the qualitative metabolite profile (ratio of methyl metabolites to TGN) that we measured after mercaptopurine and thioguanine incubation in TPMT+ cells is much closer to the ratios observed in vivo in patients with wild-type TPMT after mercaptopurine (9) and thioguanine (27) incubation than those observed with our MOCK cells (Fig. 4), suggesting that the results presented here have some applicability to the clinic.

Mercaptopurine is converted by HPRT into TIMP and subsequently to TGMP by two steps involving inosine monophosphate dehydrogenase and guanosine monophosphate synthetase. Thioguanine, however, is directly converted into TGMP and then into the TGNs by adenosine kinase (Fig. 1; Ref. 2). TIMP and TGMP exhibit similar K_{m}S for human TPMT (7), but as is true in patients with wild-type TPMT, we found that MeMPN predominated over TGNs after incubation with mercaptopurine, whereas TGN predominated over MeTGN after incubation with thioguanine. As expected, the TPMT+ cells had 9–10-fold higher cytosolic concentrations of methylated nucleotides (MeTGN or MeMPN) compared with the MOCK cells. This was associated with lower concentrations of the TINs (with mercaptopurine treatment) and TGNs (with thioguanine treatment). However, the TGN concentrations were similar between the MOCK and the TPMT+ cells after 24 h of incubation with mercaptopurine. This could be attributable to the high concentrations of the TGN, which far exceeded the capacity for the TPMT to methylate TIMP and thus left the substrate available for the inosine monophosphate dehydrogenase. Indeed, after 48 h of incubation with mercaptopurine, we observed significantly lower TGN concentrations in TPMT+ cells compared with MOCK cells.

TPMT+ cells produced higher MeMPN levels and were more sensitive to the effects of mercaptopurine (lower IC_{50} and lower viability at 48 h) compared with cells with lower TPMT. This was associated with a faster and more potent inhibition of DNPS and a

**Fig. 6.** DNPS inhibition by thioguanine (TG) and mercaptopurine (MP) in CEM cells. A, CEM-MOCK (■) and CEM-TPMT+ (■) cells were treated with 10 μM MP, and DNPS inhibition was determined at 12, 24, and 48 h. Results are expressed as the average remaining DNPS compared with untreated controls. Significantly greater inhibition of DNPS was observed in the TPMT+ cells at 12 h (P < 0.02) and at 24 h (P < 0.01) compared with MOCK cells. No differences were observed at 48 h of treatment (P = 0.10). B, CEM-MOCK (■) and CEM-TPMT+ (■) cells were treated with 1 μM TG, and DNPS was determined at 24 and 48 h. At each time point, greater inhibition of the DNPS was observed in the MOCK cells compared with TPMT+ cells (P < 0.05).
Fig. 7. DNPS inhibition versus MeMPN concentrations in CEM cells. Relationship between DNPS inhibition and concentration of MeMPNs after mercaptopurine treatment. MeMPN concentrations were measured concurrently with DNPS at 12, 24, and 48 h in the MOCK and the TPMT+ cells. Each point corresponds to the average concentrations for both MeMPN (pmol/5 × 10^6 cells) and average percentage of DNPS remaining (versus untreated controls). □, experiments with MOCK cells; ■, experiments with TPMT+ cells.

lower percentage of incorporation of deoxythioguanosine into genomic DNA. Thus, our results suggest that MeMPNs contribute to the cytotoxic effects of mercaptopurine.

There are some in vitro and in vivo data suggesting that methylation of thiouracils may contribute to their cytotoxic or adverse effects. The inhibition of mitogen stimulation in lymphocytes by mercaptopurine was shown to be greater in cells with high TPMT activity (28). Methylmercaptopurine riboside was evaluated as an anticancer drug in its own right (29), has cytotoxic effects in vitro, and synergizes with thioguanine (30). MeMPNs are potent inhibitors of phosphoribosylpyrophosphate amidotransferase (the first enzyme in DNPS), with MeTGNs being less potent inhibitors of DNPS (31). The concentration of MeMPNs producing 50% inhibition of DNPS in our in vitro model (i.e., 224 pmol/5 × 10^6 cells) is often achieved in vivo in patients having wild-type TPMT activity and receiving oral mercaptopurine (i.e., up to 375 pmol/5 × 10^6 red blood cells; Ref. 32). Thus, it seems likely that significant DNPS inhibition by MeMPNs can be achieved in vivo after oral mercaptopurine and can contribute to the effect of the drug. Methylation of mercaptopurine could also contribute to its effect through the inhibition by methylmercaptopurine riboside of nerve growth factor-activated protein kinase N (33) and angiogenic factors (34). Our results are also consistent with clinical observations. MeMPNs are produced in vast excess compared with TGN in patients with wild-type TPMT activity who received mercaptopurine (32). Although TPMT-deficient patients must be treated with markedly lower doses of mercaptopurine to avoid toxic concentrations of TGN (11), these patients do not form MeMPNs and can tolerate considerably higher TGN concentrations than patients with high TPMT activity (9, 10, 15). Interestingly, patients receiving thioguanine tolerate much higher TGN concentrations than patients receiving mercaptopurine (27); patients treated with thioguanine form no MeMPNs and exhibit a much lower MeTGN/TGN ratio than the MeMPN/TGN ratio achieved with chronic mercaptopurine (9, 27). Together, these data indicate that mercaptopurine could be considered as a “two in one” drug (35), providing a source of TGNs and MeMPNs, and both compounds could contribute to the antiproliferative effects. Two reports (36, 37) have suggested that low TPMT might predispose to the occurrence of secondary acute myeloblastic leukemia after treatment with mercaptopurine, and one of these reports (37) related its risk to either high TGN or high MeMPN concentrations. Thus, there are preclinical and clinical data supporting our finding that MeMPNs are active compounds and should be taken into account during the treatment of acute lymphoblastic leukemia.

Thioguanine treatment in MOCK and TPMT+ cells yielded oppos-
thioguanine is not superior to mercaptopurine when compared in a population of patients having a polymorphic range of TPMT activities. In addition, 90% of patients are homozygous at the TPMT locus and thus have normal or high TPMT activity. Thus, the administration of mercaptopurine in these patients could present an advantage compared with thioguanine because mercaptopurine provides a source of active MeMPNs, which could compensate for the lower TGN levels observed in patients with high TPMT. In contrast, thioguanine administration would be predicted to produce variable responses because of its susceptibility to the highly polymorphic TPMT and the lack of any offsetting activation to MeMPNs.

In conclusion, our results provide new insights into the mechanism of cytotoxicity of thiopurines and suggest a rationale for the choice of mercaptopurine over thioguanine in the treatment of acute lymphoblastic leukemia.

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