Differential Effects of Targeted Disruption of Thiopurine Methyltransferase on Mercaptopurine and Thioguanine Pharmacodynamics

Christine Hartford,¹² Erick Vasquez,¹ Matthias Schwab,¹,⁷ Mathew J. Edick,¹,⁶ Jerold E. Rehg,³ Gerard Grosveld,¹ Ching-Hon Pui,²,⁵,⁶ William E. Evans,¹,⁶ and Mary V. Relling¹,⁶

Departments of 1Pharmaceutical Sciences, 1Hematology-Oncology, 1Pathology, and 1Genetics, and 1Hematologic Malignancies Program, St. Jude Children’s Research Hospital, 332 North Lauderdale, Memphis, TN 38105-2794. University of Tennessee, Memphis, Tennessee; and 7Dr. Margarete Fischer-Bosch Institute of Clinical Pharmacology, Stuttgart, Germany, and University of Tuebingen, Tuebingen, Germany

Abstract

The recessive deficiency in thiopurine methyltransferase (TPMT), caused by germ-line polymorphisms in TPMT, can cause severe toxicity after mercaptopurine. However, the significance of heterozygosity and the effect of the polymorphism on thioguanine or in the absence of thiopurines is not known. To address these issues, we created a murine knockout of Tpmt. Pharmacokinetic and pharmacodynamic studies of mercaptopurine and thioguanine were done in Tpmt⁻/⁻, Tpmt⁺⁻, and Tpmt⁺/+ mice and variables were compared among genotypes. Methylated thiopurine and thioguanine nucleotide metabolites differed among genotypes after treatment with mercaptopurine (P < 0.0001 and P = 0.044, respectively) and thioguanine (P = 0.011 and P = 0.002, respectively). Differences in toxicity among genotypes were more pronounced following treatment with 10 daily doses of mercaptopurine at 100 mg/kg/d (0%, 68%, and 100% 50-day survival; P = 0.0003) than with thioguanine at 5 mg/kg/d (0%, 33%, and 50% 15-day survival; P = 0.07) in the Tpmt⁻/⁻, Tpmt⁺⁻, and Tpmt⁺/+ genotypes, respectively. Myelosuppression and weight loss exhibited a haploinsufficient phenotype after mercaptopurine, whereas haploinsufficiency was less prominent with thioguanine. In the absence of drug challenge, there was no apparent phenotype. The murine model recapitulates many clinical features of the human polymorphism; indicates that mercaptopurine is more affected by the TPMT polymorphism than thioguanine; and provides a preclinical system for establishing safer regimens of genetically influenced antileukemic drug therapy. [Cancer Res 2007;67(10):4965–72]

Introduction

One of the clearest examples of a pharmacogenetic polymorphism affecting drug efficacy and toxicity in humans is that caused by thiopurine methyltransferase (TPMT) deficiency (1–5). Thiopurines (mercaptopurine, thioguanine, and azathioprine) are commonly used as antineoplastics and immunosuppressants, but their proper use remains uncertain and is complicated by the TPMT polymorphism. Inherited as an autosomal codominant trait (6–8), TPMT activity in erythrocytes (reflective of other tissues as well; refs. 7, 9–11) exhibits a trimodal population frequency distribution (12–14). TPMT activity is inversely related to the concentration of active thioguanine metabolites after administration of thiopurines (4). The rare homozygous deficient individuals develop extreme myelosuppression, which can be fatal when given usual doses of thiopurines (15), necessitating a 10-fold dosage decrease to prevent such toxicity (16). Heterozygotes, constituting 10% of the population, often exhibit an intermediate degree of toxicity following mercaptopurine (17), although the role of heterozygosity in dosing thiopurines is somewhat controversial. Patients with homozygous wild-type (high) activity may be at increased risk of poor treatment response (e.g., persistence of relapse; ref. 18).

Due to the inherent toxicity and possible carcinogenicity of thiopurines (19–21), conducting randomized clinical trials to fully explore how best to use these agents in humans has ethical constraints. For example, there is considerable interest in studying the relative importance of the TPMT polymorphism for thioguanine versus for mercaptopurine, particularly because recent clinical trials have suggested a lower relapse rate with thioguanine treatment in childhood acute lymphoblastic leukemia (ALL). TPMT activates the mercaptopurine riboside intermediate metabolite of mercaptopurine, but this methylated metabolite is not present after administration of thioguanine (22). Thus, it would be useful to directly compare the effect of the TPMT polymorphism on the pharmacokinetics and pharmacodynamics of thioguanine versus mercaptopurine (3, 23). In addition, there has been much speculation about whether the polymorphism in TPMT has any biological significance in the absence of thiopurine challenge (2, 24).

In humans, the molecular basis of the TPMT polymorphism is largely related to three common nonsynonymous coding single-nucleotide polymorphisms (13), each of which renders the protein unstable (25) and subject to enhanced ubiquitination and degradation (26, 27). Thus, the homozygous deficiency in humans is characterized by almost undetectable levels of TPMT protein; heterozygotes have intermediate protein and activity levels; and homozygous wild-type individuals have high levels of protein and activity (28, 29).

We created a murine model to allow the controlled study of the TPMT polymorphism. This model has allowed us to compare the pharmacokinetics and pharmacodynamics of thioguanine and mercaptopurine in an in vivo isogenic system and to show that biological consequences of the TPMT polymorphism are evident only in the presence of drug challenge.

Note: C-H. Pui is an American Cancer Society Professor.

Requests for reprints: Mary V. Relling, Department of Pharmaceutical Sciences, St. Jude Children’s Research Hospital, 332 North Lauderdale, Memphis, TN 38105-2794. Phone: 901-495-2348; Fax: 901-525-6869; E-mail: mary.relling@sjdude.org.

©2007 American Association for Cancer Research.
doi:10.1158/0008-5472.CAN-06-3508
Materials and Methods

Chemicals. Mercaptopurine, thioguanine, 2-deoxyguanosine, 6-thioguanosine, 6-mercaptopurine riboside, and 6-methylmercaptopurine riboside were purchased from Sigma. Alkaline phosphatase was purchased from Promega. 6-Thio-2-deoxyguanosine was purchased from R.L. Chemical, Inc. All other chemicals and reagents were of high-performance liquid chromatography grade. A folic acid–deficient purified diet for mice was purchased from TestDiet. Methotrexate and trimethoprim/sulfamethoxazole (Sulfatrim pediatric suspension) were purchased from Alpharma.

Targeting construct design and generation. A clone containing the murine Tpmt gene was identified by screening a 129/Ola murine genomic DNA library, packaged in a P1 artificial chromosome vector (Genome Systems), with the assistance of Dr. Evgeny Krynetski and Dr. Mike Fessing (30, 31). The identified clone was verified to contain the Tpmt genomic locus by Southern blotting, sequencing, and fluorescent in situ hybridization. To generate the targeting construct, an 8.7-kb KpnI restriction endonuclease fragment encompassing part of exon III through a portion of intron 6 was subcloned into the pZEO-2 vector (Invitrogen). A herpes simplex virus-thymidine kinase gene cassette was cloned into the HindIII site of the pZEO vector at the S' end of the Tpmt insert, and a 2.8-kb PacI/XmaIII fragment, internal to the 8.7-kb KpnI fragment and containing exon V and VI, was replaced with a neomycin phosphotransferase gene cassette.

Generation of targeted mice. E14 embryonic stem cells provided by the laboratories of Dr. James Ihle and Dr. Gerard Grosveld (St. Jude Children’s Research Hospital) were electroporated in the presence of linearized Tpmt targeting vector DNA. After selection in G418 for 9 days, three successfully targeted clones (4B9, 7C10, and 5G3) were identified by PCR and confirmed by Southern blotting. C57BL/6 pseudopregnant mice were recipients of blastocyst injections and embryo transfer. Four germ-line transmitting chimeric mice were produced (three from clone 5G3 and one from clone 7C10). Heterozygous Tpmt (Tpmt+/−) chimeric mice produced offspring in the expected Mendelian distribution, indicating no in utero lethality due to loss of one or both functional Tpmt alleles. Because C57BL/6 have low constitutive TPMT activity (32, 33), the mixed background 129/C57BL/6 chimeric mice were backcrossed into constitutively high TPMT activity strains (129P1/ReJ, NMRI, 129X1/SvJ, and C3H/HeJ) for five generations to produce ~97% strain purity. All strains were generated from 5G3 clones. In addition, a strain of NMRI was also generated from clone 7C10. All experiments involving the production and use of the Tpmt knockout mouse described herein were approved by the Institutional Animal Care and Use Committee of St. Jude Children’s Research Hospital.

Histologic evaluation. Histologic specimens were prepared from 6-month-old mice of both genders and three genotypes (Tpmt+/+, Tpmt−/−, and Tpmt−/−) for screening of any pathologic changes associated with the Tpmt-deficient genotype. All organs were dissected and fixed in 10% neutral buffer formalin. Paraffin-embedded sections (5 μm) were visualized by H&E staining.

TPMT activity. Blood (1 mL) was collected via heart puncture into tubes containing sodium heparin. Erythrocyte lysates were prepared and analyzed for TPMT activity by the nonchelated radiochemical assay of Szumlanski et al. (34). The assay is based on the conversion of mercaptopurine to thiopurine bases and nucleosides were measured by high-pressure liquid chromatography in erythrocytes, plasma, and bone marrow cells before and after conversion with acid phosphatase, as described (36, 37). Thioguanosine nucleotides were measured in erythrocytes using a modification of a prior method (38).

Thiopurine pharmacology in children. To assess the extent to which murine data mirrored clinical data, we measured erythrocyte TPMT activity, thiopurine metabolites, and toxicity (assessed as the time-dependent requirement for a thiopurine dose decrease in children treated for ALL, as we reported; refs. 17, 39). All clinical studies were approved by our institutions’ institutional review boards, and informed consent was obtained from parents or guardians.

To evaluate chronic toxicity that partially mimics the exposure conditions of combination drug therapy given to children with leukemia, six mice of each genotype (at 2 months of age) were placed on a folate-deficient (undetectable folate) diet (Purina) because lymphoblastic leukemia therapy consists of chronic antifolates. To mimic the fact that thiopurines are given nearly continuously (for many months), thiopurines were administered via the drinking water. For chronic mercaptopurine experiments, the drinking water contained mercaptopurine (0.01 mg/mL) and sulfamethoxazole-trimethoprim for the duration of the experiments to mimic the fact that children with leukemia usually receive this antibiotic for the duration of their therapy. Given the average oral water intake of these mice, the estimated average oral intake of mercaptopurine was 2.5 mg/kg/d. Methotrexate was administered i.p. at 1 mg/kg/wk beginning at 8 weeks of age, again to mimic the exposure of children with leukemia that accompanies chronic thiopurine therapy. Moribund mice were sacrificed. After 9 weeks of this regimen, the oral dose of mercaptopurine in all of the surviving mice was doubled to 5 mg/kg/d. Experiments for chronic toxicity after thioguanine were identical to those described above, except that mercaptopurine was replaced by thioguanine, at an estimated oral dose of 1 mg/kg/d. After 5 weeks of chemotherapy, the oral dose of thioguanine was doubled to 2 mg/kg/d in the surviving mice.

To investigate the effect of a non-thiopurine drug in different Tpmt genotypes, mice were treated with etoposide (100 mg/kg) by i.p. injection. Complete blood counts were measured before and every 3 days after treatment.

Thiopurine bases and nucleosides were measured by high-pressure liquid chromatography in erythrocytes, plasma, and bone marrow cells before and after conversion with acid phosphatase, as described (36, 37). Thioguanosine nucleotides were measured in erythrocytes using a modification of a prior method (38).
Institutional Review Board. The clinical treatment protocol was one in which 91% of the weeks of therapy consisted solely of mercaptopurine and methotrexate. TPMT genotype was assessed directly by sequencing germ-line DNA or by inference based on erythrocyte TPMT activity (10, 40). De novo purine synthesis was measured in bone marrow at diagnosis of leukemia (35).

Statistical analysis. Pharmacokinetic and toxicity data are reported as means with 95% confidence intervals (95% CI). Differences in pharmacokinetic and toxicity variables among all three genotypes were compared using the Kruskal-Wallis analysis of ranks. Where only two groups were compared, the Mann-Whitney U test was used. The Kaplan-Meier method was used to calculate survival, and differences in survival among genotypes were analyzed using the log-rank test. P < 0.05 was considered statistically significant for all analyses. All analyses were two sided.

Results

Homozygous deficient Tpmt (Tpmt−/−) mice were viable, fertile, and indistinguishable from their wild-type Tpmt (Tpmt+/+) or heterozygous Tpmt (Tpmt+/−) littermates by appearance; life span; reproductive capacity; and histology of liver, lung, kidney, stomach, duodenum, small and large intestine, spleen, thymus, lymph nodes, heart, adrenals, reproductive organs, bone marrow, and brain.

TPMT activity. Mean erythrocyte TPMT activity differed among Tpmt+/+, Tpmt+/−, and Tpmt−/− genotypes in five different strains of Tpmt knockout mice (Fig. 1). The NMRI-5G3 strain was used for all further experiments. The magnitude of differences in TPMT activity among Tpmt genotypes in mice was similar to that measured in 152 children with leukemia (Fig. 1; ref. 17).

Endogenous purines. To assess possible effects of TPMT in the absence of exogenous thiopurine exposure, we documented that the mean rate of de novo purine synthesis in bone marrow cells did not differ among Tpmt+/+, Tpmt+/−, and Tpmt−/− genotypes [3.00 (95% CI, 2.89–3.10), 3.44 (95% CI, 2.11–4.76), and 3.43 (95% CI, 2.59–4.27) pmol newly synthesized purines/nmol unlabeled purines/h, respectively; P = 0.35]. Likewise, the median rates of

Figure 2. A, TGN levels in bone marrow cytosol differed by genotype. TGN levels were measured in Tpmt mice 4 h after mercaptopurine (50 mg/kg; top) and thioguanine (50 mg/kg; bottom) treatments. Inset, clinical data for steady-state erythrocyte TGN levels from patients with leukemia. B, erythrocyte MeTIMP levels differed by genotype. MeTIMP levels were measured in Tpmt mice after mercaptopurine treatment (50 mg/kg; top) and MeTGN levels were measured after thioguanine treatment (50 mg/kg; bottom). Inset, clinical data for MeTIMP levels in patients (www.pharmgkb.org PS206669; ref. 17). Boxes, 25th, 50th, and 75th percentiles; whiskers, 5th and 95th percentiles. P values were determined by the Kruskal-Wallis test.
de novo purine synthesis did not differ (\( P = 0.68 \)) in bone marrow leukemia cells obtained at diagnosis of B-lineage ALL among 105 children who were homozygous for wild-type TPMT (0.91; 95% CI, 5.10–5.82 pmol/nmol/h) compared with the 11 patients with heterozygous TPMT genotype (0.88; 95% CI, 4.12–8.58 pmol/nmol/h). Thus, targeted disruption of Tpmt did not have obvious effects on purine homeostasis.

**Pharmacokinetics.** Thioguanine nucleotide (TGN) concentrations in bone marrow cytosol differed among Tpmt+/+ and Tpmt+/– genotypes after treatment with both mercaptopurine [3.24 (95% CI, 2.59–3.88), 3.58 (95% CI, 2.40–4.76), and 5.13 (95% CI, 3.84–6.42) pmol/5 \( \times 10^9 \) cells, respectively; \( P = 0.044 \)] and thioguanine [99.7 (95% CI, 76.0–123), 143 (95% CI, 109–177), and 216 (95% CI, 158–274) pmol/5 \( \times 10^9 \) cells, respectively; \( P = 0.002 \); Fig. 2A]. We compared the Tpmt+/– genotype with both Tpmt+/+ and Tpmt+/– genotypes to evaluate whether there was an effect of haploinsufficiency. After treatment with mercaptopurine, there was no significant difference in TGN concentration in bone marrow cytosol between Tpmt+/– and either Tpmt+/+ (\( P = 0.67 \)) or Tpmt+/– (\( P = 0.09 \)) genotypes. After thioguanine treatment, the difference in TGN concentration between Tpmt+/+ and Tpmt+/– did not reach statistical significance (\( P = 0.07 \)), but the difference between Tpmt+/+ and Tpmt+/– genotypes was significant (\( P = 0.041 \)). Consistent with clinical data (41, 42), the concentration of TGNs was much higher after thioguanine than after mercaptopurine treatment [mean, 99.7 (95% CI, 76.0–123) versus 3.24 (95% CI, 2.59–3.88) pmol/5 \( \times 10^9 \) cells for Tpmt+/+ and 216 (95% CI, 158–274) versus 5.13 (95% CI, 3.84–6.42) pmol/5 \( \times 10^9 \) cells for Tpmt+/– mice, respectively].

After treatment with mercaptopurine (50 mg/kg), there was a significant difference (\( P < 0.0001 \)) in the erythrocyte concentration of methylthioinosine monophosphate (MeTIMP) among genotypes, with Tpmt+/+ mice having the highest concentration (mean, 1,104; 95% CI, 902–1,306 pmol/8 \( \times 10^8 \) cells), Tpmt+/– mice having intermediate concentration (mean, 755; 95% CI, 568–942 pmol/8 \( \times 10^8 \) cells; \( P = 0.015 \)), and Tpmt+/– mice having no measurable MeTIMP (Fig. 2B). The methylated metabolites after thioguanine (50 mg/kg) revealed a significant difference in the erythrocyte methylthioguanine nucleotide (MecTG) concentrations (\( P = 0.011 \)) among the three genotypes, although the difference between Tpmt+/+ and Tpmt+/– mice was not significant [135 (95% CI, 66.0–204) versus 166 (95% CI, 72–259) pmol/8 \( \times 10^8 \) cells, respectively; \( P = 0.34 \); Fig. 2B]. As has been observed in patients (41), the concentration of the relevant methyl metabolites was much higher after mercaptopurine than after thioguanine treatment [mean, 1,104 (95% CI, 902–1,306) versus 135 (95% CI, 66–204) pmol/8 \( \times 10^8 \) cells among Tpmt+/+ mice, respectively].

Clinical pharmacokinetic data from patients undergoing treatment for ALL were similar to those obtained from the mice. The mean levels of MeTIMP were 17,363 (95% CI, 14,543–20,184) versus 8,688 (95% CI, 1,244–18,619) versus 0.05 pmol/8 \( \times 10^8 \) cells in TPMT+/+, TPMT+/–, and TPMT+/– patients, respectively (\( P = 0.007 \); Fig. 2B). Similarly, there was a significant difference among the genotypes in the levels of TGNs: 2.61 (95% CI, 2.43–2.78) versus 6.02 (95% CI, 3.60–8.43) versus 22.28 (95% CI, 49.79–94.3) pmol/5 \( \times 10^8 \) cells in TPMT+/+, TPMT+/–, and TPMT+/– patients, respectively (\( P < 0.001 \); Fig. 2A).

**Toxicity.** After 10 days of treatment with mercaptopurine (100 mg/kg/d i.p.) as a single agent, significant differences were observed among the genotypes in survival, weight, complete blood counts, and serum aspartate aminotransferase levels (Figs. 3 and 4).

Heterozygotes had greater mean weight loss [−9.22 (95% CI, −15.0 to −3.45) versus −1.26 (95% CI, −5.02 to 2.51) g; \( P = 0.025 \)] and more anemia [5.67 (95% CI, 4.86–6.85) versus 9.91 (95% CI, 7.73–12.10) g/dL; \( P = 0.01 \)] than Tpmt+/+ littersmates. Heterozygotes also had higher mean platelet counts [264 (95% CI, 174–299)] versus 101 (95% CI, 70.7–131) \( \times 10^9 \) /µL; \( P = 0.006 \)], greater mean aspartate aminotransferase levels [82.2 (95% CI, 66.5–97.8) versus 51.8 (95% CI, 34.8–68.8) units/L; \( P = 0.023 \)], and longer survival (\( P = 0.005 \)) than Tpmt+/– littersmates. Thioguanine was more potent than mercaptopurine; preliminary experiments with 50, 25, and 10 mg/kg/d were all quite toxic to all three genotypes. At 5 mg/kg/d of thioguanine i.p. as a single agent, all mice survived for the 10 days of treatment with the exception of one Tpmt+/– mouse, but all three genotypes experienced profound myelosuppression and there were no differences in any of the hematologic variables measured among the genotypes or in overall survival (Figs. 4 and 5). When comparing Tpmt+/– with either Tpmt+/+ or Tpmt+/– genotype at this dose, only platelet count [115 (95% CI, 88.8–140) versus 81.5 (95% CI, 62.9–100) \( \times 10^9 \) /µL; \( P = 0.048 \)] and percent weight loss [−3.46 (95% CI, −6.90 to −1.60) versus −22.5 (95% CI, −26.2 to −19.5) g; \( P = 0.014 \)] differed significantly between Tpmt+/+ and Tpmt+/– genotypes, respectively. We tested an even lower dose of thioguanine (2.5 mg/kg/d) and similarly found evidence of myelosuppression in all genotypes. There was a significant
difference, however, in all complete blood count variables measured as well as in percent weight loss when comparing $Tpmt^{+/-}$ and $Tpmt^{-/-}$ genotypes, with $Tpmt^{-/-}$ mice being more profoundly affected (data not shown).

Thiopurines are often administered chronically as daily oral doses and combined with weekly methotrexate to treat ALL. We assessed the effects of chronic exposure by treating mice with daily oral mercaptopurine (2.5 mg/kg/d) or thioguanine (1 mg/kg/d) combined with weekly i.p. methotrexate (1 mg/kg). For both thiopurines, all of the $Tpmt^{-/-}$ mice were sacrificed due to toxicity after 4 to 8 weeks, with little toxicity observed in the $+/-$ and $+/+$ mice. Therefore, to hasten the onset and to increase the probability of toxicity in the surviving mice, the mercaptopurine dose was increased from 2.5 to 5 mg/kg/d beginning at 9 weeks, and the thioguanine dose was increased from 1 to 2 mg/kg/d at 5 weeks, leaving the methotrexate and trimethoprim/sulfamethoxazole doses unchanged. In this setting, survival of $Tpmt^{-/-}$ mice was 0% at 8 weeks following mercaptopurine and 0% at 4 weeks following thioguanine treatment, both being significantly lower than their respective $Tpmt^{+/-}$ or $Tpmt^{+/-}$ littermates ($P = 0.045$; no $Tpmt^{-/-}$ mice survived to this time point. Interestingly, mean aspartate aminotransferase levels did not differ among genotypes after 10 days of thioguanine [67.00 (95% CI, 42.57–91.43), 60.6 (95% CI, 30.52–90.68), and 62.25 (95% CI, 25.17–99.33) units/L in

Myelosuppression (measured by the absolute neutrophil count, hemoglobin, and platelet count) was significantly different among $Tpmt^{+/-}$, $Tpmt^{-/-}$, and $Tpmt^{-/-}$ genotypes after the first 4 weeks of chronic oral mercaptopurine combined with methotrexate (data not shown). The poor survival rates precluded similar analyses with chronic oral thioguanine/methotrexate treatment.

Hepatotoxicity is a known adverse effect of thiopurine therapy (43–45). In contrast to myelosuppression after mercaptopurine, it seems to occur more commonly among patients with high rather than low TPMT activity (46, 47). In mice, after acute exposure to mercaptopurine alone (100 mg/kg/d), serum aspartate aminotransferase levels were related to the number of wild-type copies of $Tpmt$ ($P = 0.014$; Fig. 3), with heterozygotes having higher aminotransferase levels than $Tpmt^{-/-}$ mice [80.2 (95% CI, 56.2–104) versus 51.8 (95% CI, 34.8–68.8) units/L, respectively, $P = 0.022$]. A similar relationship to hepatotoxicity was observed after chronic exposure to 8 weeks of oral mercaptopurine combined with methotrexate, with aspartate aminotransferase levels higher in $Tpmt^{+/-}$ than in $Tpmt^{-/-}$ littermates [126 (95% CI, 103–148) versus 91.17 (95% CI, 60.94–121.39) units/L, respectively, $P = 0.045$; no $Tpmt^{-/-}$ mice survived to this time point. Interestingly, mean aspartate aminotransferase levels did not differ among genotypes after 10 days of thioguanine [67.00 (95% CI, 42.57–91.43), 60.6 (95% CI, 30.52–90.68), and 62.25 (95% CI, 25.17–99.33) units/L in

Figure 4. Cumulative proportion of $Tpmt$ mice surviving after exposure to mercaptopurine (100 mg/kg/d; top) or thioguanine (5 mg/kg/d; bottom). $P$ values were determined by the log-rank test.

Figure 5. Blood counts, serum aspartate aminotransferase levels, and percentage of body weight loss of $Tpmt$ mice after 10 d of treatment with thioguanine as a single agent (5 mg/kg/d i.p.). Boxes, 25th, 50th, and 75th percentiles; whiskers, 5th and 95th percentiles. $P$ values were determined by the Kruskal-Wallis test.
Tpmt+/+, Tpmt+/-, and Tpmt-/-, respectively; P = 0.53; Fig. 5); poor survival precluded a similar comparison after chronic exposure to oral thioguanine combined with methotrexate.

Etoposide as a negative control did not result in any differences in complete blood counts or in survival (P = 0.49) among genotypes (data not shown).

Discussion

We created a murine model that recapitulates all the known elements of the human genetic polymorphism in TPMT. This model therefore allowed us to address fundamental questions about the effects of TPMT on thiopurine dosing.

When thiopurines are used to treat cancer, they are almost always used in a multidrug context. Because of overlapping toxicities among anticancer drugs, it is often not possible to discern which agent is the primary culprit causing myelosuppression using a trial-and-error approach in the clinic. Therefore, the diagnosis of a defect in TPMT can serve as a clinically useful basis on which to target thiopurines as the likely cause of myelosuppression in the setting of multiagent chemotherapy. The clinical data supporting an increased risk of thiopurine-induced myelosuppression associated with the relatively rare homozygous deficiency are strong, with a >10-fold dosage reduction required in such patients (44). Our murine data are consistent with a dramatic effect of homozygous defects of TPMT. The magnitude of the effect of heterozygosity and its implications for dose reductions clinically are less clear (17). Herein, with acute administration of thiopurines alone, we showed that heterozygosity affects mercaptopurine-induced myelosuppression (Fig. 3), but haploinsufficiency was more difficult to show after thioguanine treatment (Fig. 5). With chronic thiopurine exposures, and in the presence of methotrexate (mimicking the clinical regimen used for treatment of ALL), toxicity after mercaptopurine was only slightly greater in Tpmt+/- mice than in Tpmt+/+ mice.
The Tpmt activity differences among the genotypic groups were identical to those observed in patients (Fig. 1) and clear evidence for haploinsufficiency was conferred by heterozygosity, as is true in humans (40). Thus, the model appears to be suitable for addressing thiopurine questions that cannot be feasibly tested in humans. Our data indicate that toxicity differences among patients of differing TPMT genotypes are likely due to differences in metabolism of thiopurines and not to any underlying susceptibility caused by TPMT variation. There was no evident phenotype produced by abrogation of the Tpmt gene in the absence of drug challenge. In this regard, we tested for more subtle biochemical effects of Tpmt deficiency by measuring constitutive levels of de novo purine synthesis and found no difference among the Tpmt+/+ , Tpmt+/− , or Tpmt−/− genotypes. Because TPMT uses S-adenosylmethionine as a cofactor, which plays a crucial role in purine and pyrimidine homeostasis, these findings indicate that TPMT is unlikely to play an important role in endogenous purine synthesis. Moreover, as expected, there were no differences in toxicity among genotypes after treatment with etoposide, an agent that is not metabolized by TPMT.

Methylated active metabolites of mercaptopurine (MeTIMP) differed among Tpmt genotypes, mirroring the differences observed among patients (Fig. 2B). MeTIMP concentrations are much higher, relative to dose or to active TGN metabolites, following mercaptopurine than are the analogous methylated metabolites (MeTGN) after thioguanine (Fig. 2B). Interestingly, haploinsufficiency was not evident for production of MeTGN after thioguanine as it was for production of MeTIMP after mercaptopurine (Fig. 2B), which is consistent with haploinsufficiency being more evident for toxicity after mercaptopurine than thioguanine. Active TGN metabolites differed by Tpmt genotype after mercaptopurine and after thioguanine, as was anticipated from clinical data (41).

In humans, thioguanine and mercaptopurine show substantially different pharmacologic profiles, despite sharing common TGN metabolites. Mercaptopurine is more subject to methylation than to the inactive base and the active MeTIMP than is thioguanine, for which anabolism to TGNs plays a greater quantitative role. The difficulty in discerning acute toxicity differences among TPMT genotypic groups with thioguanine alone may be partly because it was difficult to find a dose low enough that was not toxic in all three genotypes; however, it is consistent with the extensive TGN formation after thioguanine compared with mercaptopurine.

Thiopurines are associated with hepatotoxicity; hepatic transaminase elevations have been related to MeTIMP concentrations after mercaptopurine (48) whereas hepatic veno-occlusive disease is more common after thioguanine than mercaptopurine and seems to be dose related (49). Our data are consistent with the hypothesis that TPMT may contribute to hepatocellular toxicity via methylation of mercaptopurine metabolites (43, 45, 50) but does not relate to thioguanine hepatotoxicity; we did not observe transaminasemia after thioguanine regardless of Tpmt genotype. The clinical hepatic toxicity due to thioguanine is likely due to an independent mechanism. At necropsy, no hepatic pathology was observed after thioguanine treatment with the regimens used herein.

In summary, the targeted disruption of the Tpmt gene recapitulates the essential elements of the human pharmacogenetic polymorphism, both pharmacokinetically and pharmacodynamically, but the model also further differentiates specific adverse and pharmacologic effects of thioguanine versus mercaptopurine. Such murine models of human germ-line genetic variability provide a laboratory basis for the systematic optimization of dosages of anticancer medications.

Acknowledgments

Received 9/27/2006; revised 1/12/2007; accepted 3/7/2007.

Grant support: National Cancer Institute grants T32 CA070089, CA 51001, CA 36401, and CA21765; NIH/National Institute of General Medical Sciences Pharmacogenetics Research Network and Database, grants U01 GM61393 and U01 GM61374 (http://pharmgkb.org/); a Center of Excellence grant from the State of Tennessee; American Lebanese Syrian Associated Charities; and the Robert-Bosch Foundation, Stuttgart, Germany.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank our clinical staff, research nurses, and patients and their parents for participation in this study; Paxton Baker, Yaqin Chu, May Chung, Nancy Duran, Natalya Lenchik, Margaret Needham, and Emily Melton for outstanding technical assistance; and Nancy Kornegay and Mark Wilkinson for computer and database expertise.

References

29. Szumlanski C, Otteness D, Her C, et al. Thiopurine methyltransferase pharmacogenetics: human gene clon-
Differential Effects of Targeted Disruption of Thiopurine Methyltransferase on Mercaptopurine and Thioguanine Pharmacodynamics

Christine Hartford, Erick Vasquez, Matthias Schwab, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/67/10/4965

Cited articles
This article cites 50 articles, 10 of which you can access for free at:
http://cancerres.aacrjournals.org/content/67/10/4965.full.html#ref-list-1

Citing articles
This article has been cited by 2 HighWire-hosted articles. Access the articles at:
/content/67/10/4965.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.