Tissue Specific Toxicities of the Anticancer Drug 6-Thioguanine Is Dependent on the Hprt Status in Transgenic Mice

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ABSTRACT

6-Thioguanine (6TG) a cytostatic antimetabolite is currently used to treat patients with cancer, in particular leukemias. However, one drawback of such use is the development of 6TG resistance. Hypoxanthine-guanine phosphoribosyl transferase (Hprt) plays a crucial role in the bioactivation of 6TG. Loss of Hprt has been associated with the resistance of leukemias to 6TG chemotherapy, however, nothing has been known about the effect of Hprt status on tissue specific toxicity of 6TG in vivo. We determined the effect of Hprt status on the tissue-specific toxicity of 6TG in vivo in transgenic Hprt-deficient mice. The approximate lethal dose for Hprt-deficient mice was 23-fold higher than for the wild-type. Serum biochemical analyses of 6TG-treated wild-type mice showed elevated serum enzyme levels characteristic of liver damage whereas the levels in Hprt-deficient 6TG-treated mice were within normal physiological limits. Histopathological examination of tissues from wild-type and from Hprt-deficient mice showed contrasting spectrums of microscopic lesions. Wild-type mice had loss of hematopoietic cells from bone marrow starting at the lowest dose of 25 mg/kg 6TG whereas Hprt-deficient mice had normal bone marrow and spleen even at doses of 720 mg/kg 6TG. Wild-type mice also experienced severe loss of epithelial cells from the gastrointestinal tract starting at 50 mg/kg; however, the gastrointestinal tract of Hprt −/− mice remained unaffected. Wild-type livers revealed atrophy and necrosis at doses of 25 mg/kg 6TG although Hprt −/− livers displayed no effect until 507 mg/kg. In this study we show that Hprt-deficient mice had 6TG-resistant bone marrow and there are several other factors contributing to 6TG resistance in patients. Because variations among people exist in terms of their 6TG sensitivity, determining 6TG sensitivity of lymphocytes prior to 6TG chemotherapy and restricting treatment to 6TG-sensitive patients may improve the efficacy.

Cytotoxic drug resistance is a major obstacle to successful chemotherapy in cancer patients. 6TG is effective as leukemia treatment agent as well as immunosuppressant (Calabresi and Parks, 1985; Loo and Nelson, 1982). It has been noted that virtually all major current protocols for “average” and “low risk” ALL include as a core component of continuing chemotherapy daily doses of 6-mercaptopurine (6MP), an analog of 6TG that is metabolized in the same way (Lennard and Lilleyman, 1989). 6TG is first converted to 6TGMP by Hprt in the purine salvage pathway (fig. 1, (Calabresi and Parks, 1985)). The biological activity of this product is several-fold. First, 6TGMP works as a pseudofeedback inhibitor of glutamine-5-phosphoribosylpyrophosphate amidotransferase and blocks purine biosynthesis. Second, 6TGMP inhibits its IMP dehydrogenase and thus purine interconversion. The net consequence of this activity is a block of the synthesis and utilization of purine nucleotides (Calabresi and Parks, 1985). Third, 6TGMP, after conversion to the tri-phosphate form is incorporated into either DNA or RNA (LePage, 1963; Ling et al., 1992; Pan and Nelson, 1990). Single strand DNA breaks occur on the DNA strand on which guanine has been replaced by thioguanine (Pan and Nelson, 1990) probably due to blockage of strand extension because of its poor ability to act as a substrate for polymerase and DNA ligase (Ling et al., 1992). 6TG is eliminated from the body mostly in the form of inactive metabolites which include 6TX, 6TUA and 6MeTG [fig. 1; (Elion, 1967; LePage and Whitecar, 1971)]. The distribution of these metabolites seems to be different in mice and man (Elion, 1967; Elion et al., 1963; LePage and Whitecar, 1971). Several laboratory and clinical observations suggest that Hprt deficiency causes cellular resistance to 6TG. For example, cells from Lesch-Nyhan syndrome patients lack Hprt and are resistant to 6TG (Dempsey et al., 1983; Yamanaka et al.,.

ABBREVIATIONS: 6MeTG, 6-methyl-thioguanine; 6MP, 6-mercaptopurine; 6TG, 6-thioguanine; 6TGMP, 6-thioguanine monophosphate; 6TGTP, 6-thioguanine triphosphate; 6TUA, 6-thioic acid; 6TX, 6-thioxanthine; ALL, acute lymphoblastic leukemia; Hprt, hypoxanthine-guanine phosphoribosyl transferase; IMP, inosine monophosphate; LD₅₀, median lethal dose; PRPP, phosphoribosyl pyrophosphate; BUN, blood urea nitrogen; AST, aspartate amino transferase; ALT, alanine amino transferase; CK, creatine kinase; AP, alkaline phosphatase; Aprt, adenine phosphoribosyltransferase.

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As a quantitative parameter of the acute 6TG toxicity in Hprt-deficient and Hprt wild-type mice we determined the approximate lethal dose that corresponds to the LD₅₀ ± 30% for most chemicals (Deichmann and LeBlanc, 1943). Furthermore, we performed histopathological and serum biochemical analysis of treated wild-type and Hprt-deficient animals. These studies determined the effect of Hprt status on the spectrum of target organ specificity of 6TG toxicity.

Material and Methods

Animals and drug treatment. Transgenic Hprt-deficient female mice were obtained from Dr. B. Koller (University of North Carolina, Chapel Hill, NC). These mice have a 129Jd genetic background and carry a deletion of exons 1 and 2 of the Hprt gene (Hooper et al., 1987). Control animals, wild-type 129Jd mice, were obtained from the Jackson Laboratory (Bar Harbor, ME). The mice were kept in SPF conditions, provided with standard diet and water ad libitum. Animal care and experimental procedures were carried out in agreement with institutional guidelines.

6-Thioguanine (2-amino-6-merkaptopurine) was purchased from Sigma Chemical Co. (St. Louis, MO). The compound was suspended in distilled water and sonicated for 10 min before each i.p. injection. 9-ethyladenine (Sigma Chemical Co., St. Louis, MO) was dissolved in sterile saline and stored at 4°C until i.p. application.

Acute toxicity, approximate lethal dose. The approximate lethal dose of 6TG after single i.p. injections was determined (Deichmann and LeBlanc, 1943). In summary, the animals were treated with single doses of 6TG i.p. at concentrations starting at 100 mg/kg that increased by 50% for each consecutive dose. Control mice were treated with the same volume of sterile distilled water. Because the LD₅₀ is known for wild-type mice this protocol was modified for doses below 100 mg/kg, and concentrations of only 25 and 50 mg/kg were used to save animals. The lowest dose at which the first animal died was the approximate lethal dose. This dose corresponds to the LD₅₀ ± 30% for most chemicals (Deichmann and LeBlanc, 1943; Deichmann and Mergard, 1948). This protocol significantly reduces the number of experimental animals, limits unnecessary suffering and complies with current Guideline of Animal Studies (IARC, 1993).

Two sets of 5-month-old female animals were used for all acute toxicity experiments. The 6TG suspension was injected i.p. The controls were injected with sterile water. The health status of the animals was observed twice daily and body weight was measured daily for 14 subsequential days. The dead or killed moribund mice were immediately necropsied, and the organs were stored in 10% buffered neutral formalin until further analysis. All survivors were treated with the same volume of sterile distilled water. Because the LD₅₀ is known for wild-type mice this protocol was modified for doses below 100 mg/kg, and concentrations of only 25 and 50 mg/kg were used to save animals. The lowest dose at which the first animal died was the approximate lethal dose. This dose corresponds to the LD₅₀ ± 30% for most chemicals (Deichmann and LeBlanc, 1943; Deichmann and Mergard, 1948). This protocol significantly reduces the number of experimental animals, limits unnecessary suffering and complies with current Guideline of Animal Studies (IARC, 1993).

Histopathological examination and serum biochemical analysis of serum. The organs were prepared as paraffin-embedded tissue glass slides stained with hematoxylin and eosin and evaluated according to the NTP (National Toxicology Program of NIEHS) standards. A complete cross-section of each organ, when possible, was evaluated (liver, spleen, gastrointestinal tract, femoral bone marrow, mandibular and mesenteric lymph nodes, kidney, brain, uterus and ovaries, lungs and heart). For liver, two cross-sections, one of each of the two largest liver lobes were examined. For kidneys, an entire cross-section (left longitudinal, right transverse) were evaluated. Lungs had two cross-sections (one of each of the two largest lobes). The entire sections on the slides (all fields) were evaluated under blinded conditions for lesions and scored (graded) on a subjective basis compared to control animals. The grades were as follows: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked, and – = no pathological changes. The preparation and evaluation of slides used the NTP criteria and terminology (Chhabra et al., 1990).
Serum biochemistry parameters analyzed included BUN, AST, ALT, CK and AP. The analyses were performed at Tufts University Veterinary Medical Diagnosis Laboratory with the chemical analyzer Hitachi 747. Blood was collected from the posterior vena cava of Hprt deficient mice that were either treated with water, 25,500 or 720 mg/kg 6TG i.p. and wildtype mice administered 25 mg/kg 6TG.

### Results

**Acute toxicity, approximate lethal dose.** Hprt-deficient and wild-type mice tolerated a single i.p. injection of 6TG without immediate toxic symptoms or distress. The approximate lethal dose of 6TG for wild-type mice was 50 mg/kg (table 1). Hprt deficiency caused a dramatic increase in resistance to 6TG. The approximate lethal dose for \( Hprt^{-/-} \) animals was 1148 mg/kg, which represents a 23-fold increase over the wild-type strain. Lethal doses in both \( Hprot^+/+ \) and \( Hprt^{-/-} \) strains caused the same symptoms including loss of body weight, decreased activity progressing to lethargy and coma. Sublethal doses in \( Hprot^{-/-} \) animals caused no visible effects or changes of body weight. However, at the 25-mg/kg sublethal dose for the wild type mice a 7% decrease in body weight was found.

**Histopathological examination.** Complete histopathological analysis of wild-type mice and of Hprt-deficient mice was performed for all doses used for table 2 up to 720 and 2571 mg/kg, respectively. Some control Hprt-deficient mice had liver lesions characterized by moderate to marked hepatocellular fatty change typified by large distinct cytoplasmic vacuoles and centrilobular hypertrophy. These lesions did not show any correlation with 6TG dose and did not occur in wild-type control or 6TG-treated mice until lethal doses of 225 mg/kg for the fatty change and 150 mg/kg for centrilobular hypertrophy. Therefore, these lesions may be the consequence of Hprt deficiency.

Lesions seen in wild-type and \( Hprot \) mutant mice differed strikingly (table 2) for the same doses of 6TG. Starting at 6TG doses of 25 mg/kg \( Hprot \) wild-type mice had liver lesions such as necrosis of scattered individual hepatocytes and atrophy of hepatocytes typified by decreased cell size. At higher doses, we observed centrilobular hypertrophy or increased relative size of centrilobular compared to other hepatocytes.

**Table 1**

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>( Hprot^+/+ )</th>
<th>( Hprot^{-/-} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival (days)</td>
<td>Body weight* (%)</td>
<td>Survival (days)</td>
</tr>
<tr>
<td>0</td>
<td>Surv 105 ± 4</td>
<td>Surv 102 ± 4</td>
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<tr>
<td>25</td>
<td>Surv 93 ± 6</td>
<td>Surv 101 ± 4</td>
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<tr>
<td>50</td>
<td>13 ± 0.0</td>
<td>150 ± 0.0</td>
</tr>
<tr>
<td>100</td>
<td>10.5 ± 0.5</td>
<td>69 ± 7</td>
</tr>
<tr>
<td>150</td>
<td>8.5 ± 0.5</td>
<td>76 ± 3</td>
</tr>
<tr>
<td>225</td>
<td>7 ± 0.0</td>
<td>80 ± 4</td>
</tr>
<tr>
<td>338</td>
<td>4 ± 0.0</td>
<td>88 ± 5</td>
</tr>
<tr>
<td>508</td>
<td>4 ± 0.0</td>
<td>85 ± 6</td>
</tr>
<tr>
<td>720</td>
<td>4 ± 0.0</td>
<td>80 ± 6</td>
</tr>
<tr>
<td>1148</td>
<td>ND</td>
<td>8.5 ± 0.5</td>
</tr>
<tr>
<td>1714</td>
<td>ND</td>
<td>4 ± 0.0</td>
</tr>
<tr>
<td>2571</td>
<td>ND</td>
<td>4 ± 0.0</td>
</tr>
</tbody>
</table>

Mice were treated i.p. with single doses of 6TG, controls were treated with the same volume of sterile water. Body weights were measured daily for 14 days. *Percentage of the body weight on the 14th day of the body weight before the experiment; surv, animals survived the 14 days period; ND, experiment not done. The values represent averages for two animals ± range.

In contrast, Hprt-deficient mice did not show necrosis until doses of 507 mg/kg or more, and even at these higher doses necrosis was less severe. In contrast to untreated controls and treated wild-type mice, all Hprt-deficient mice treated with sublethal doses of 6TG (100–720 mg/kg) showed slight extramedullary hematopoiesis in the liver, which receded at lethal doses.

Wild-type mice even at the lowest dose of 25 mg/kg 6TG experienced depletion and necrosis of hematopoietic tissues, specifically the femoral bone marrow. In addition, cells of the marrow and splenic red pulp were absent or necrotic (table 2; fig. 2). Mandibular and mesenteric lymph nodes had depletions in cell numbers. In contrast, Hprt-deficient mice administered doses up to 720 mg/kg 6TG had normal to hypercellular bone marrow, spleen and lymph nodes (table 2; fig. 2). In fact, all Hprt-deficient mice treated with sublethal doses of 6TG from 50 to 720 mg/kg showed hypercellular bone marrow whereas none of the wild-type mice did.

Wild-type mice showed atrophy and/or necrosis of gastrointestinal epithelium (stomach, small and large intestines) such as loss of cells (ulcer/erosion) and decreased thickness of cell layers and height of villi and individual epithelial cells starting at doses of 50 mg/kg 6TG (table 2, fig. 3). In contrast, Hprt-deficient mice had normal to very minimal changes of gastrointestinal tract epithelium.

Kidneys and other tissues examined in wild-type mice were within normal limits. In contrast, 6TG-treated Hprt-deficient mice at sublethal doses (150–720 mg/kg) had kidney lesions including scattered dilated renal tubules, focal interstitial inflammation, focal tubule basophilia and some glomerulopathy characterized by increased numbers of cells (table 2).

Hprt deficient mice administered with lethal doses of 6TG (1148 mg/kg or more) had lesions of liver, spleen and bone marrow similar to those observed in the wild-type mice treated with 25 mg/kg or more (table 2). The kidney lesions in Hprt-deficient mice decreased at toxic doses (table 2).

**Serum biochemical analysis.** Hprt wild-type mice were treated with the sublethal dose of 25 mg/kg 6TG and Hprt mutant mice were treated with the sublethal doses of 25,500 and 750 mg/kg 6TG. Serum samples taken 14 days after treatment were evaluated for the levels of BUN, AST, ALT, CK and AP. Elevated BUN is associated with dehydration and/or renal insufficiency. Elevated activities of ALT and AST are characteristic for liver damage, particularly necrosis, cirrhosis and/or hepatitis and also muscle trauma or myocardial infarction or myositis. Elevated AP is associated mostly with increased bone marrow metabolism and also with hepatocellular damage during hepatitis. CK is predominantly located in muscles and therefore its increased activities are consequence of muscular trauma, myocardial infarction or myopathic disorders.

Administration of a sublethal dose of 25 mg/kg 6TG resulted in clinically significant elevated levels of AST, and ALT (table 3A) in Hprt wild-type mice suggesting hepatocellular possibly necrotic damage in those animals. Levels of CK and AP did not increase suggesting that 6TG does not cause muscular damage or bone disorders. There was about a doubling of BUN levels, slightly above the physiological range. Because we did not detect histological evidence for renal toxicity of 6TG in wild-type mice, these slightly elevated levels of BUN could be the result of dehydration caused by
impaired gastrointestinal epithelia or due to decreased fluid intake. In contrast, Hprt-deficient mice, even after sublethal doses of 720 mg/kg, showed no clinically significant changes of the serum biochemical parameters (table 3B).

**Effect of the Aprt inhibitor 9-ethyl adenine on 6TG toxicity in Hprt-deficient mice.** Aprt catalyzes phosphorylation of adenine to its monophosphate. Although this reaction is adenine specific (Blakley, 1986) it might be possible that Aprt also phosphorylates guanine (in our case 6TG) in the Hprt-deficient background in the presence of massive amounts of 6TG. Thus, it might be possible that inhibition of Aprt enzymatic activity actually decreases toxicity of 6TG in Hprt-deficient mice. It has been shown that Aprt activity is inhibited in mice by injection of 9-ethyl adenine (2.5 × 10^{-6} mol) i.p. in 48 hrs intervals (Wu and Melton, 1993).

To determine whether Aprt may activate 6-TG in Hprt-deficient mice we treated such mice with five injections of 9-ethyladenine (2.5 × 10^{-6} mol) in 48-hr intervals before 6TG application. The control groups received 9-ethyladenine or saline, respectively. Three mice were used per group. 9-ethyladenine and saline-pretreated animals received injections with the lethal dose of 6TG (1000 mg/kg). One group of 9-ethyladenine-treated animals served as a control for possible side effects of Aprt inhibition. The treatment with 9-ethyladenine alone did not result in any apparent toxic symptoms. All 6TG-treated animals including 9-ethyladenine as well as saline pretreated mice showed the same symptoms of 6TG toxicity that resulted in coma and death 5 days after the 6TG injection. Thus, 9-ethyladenine pretreatment did not protect Hprt-deficient animals against 6TG toxicity suggesting that Aprt may not be responsible for the remaining toxicity of 6TG in Hprt-deficient mice.

**Discussion**

6TG has been used for the treatment of leukemias and as an immunosuppressive agent for several decades. However, to our knowledge little or nothing is known about the effect of Hprt status on the tissue-specific toxicity of 6TG in animals. Thus, we determined the effect of Hprt status on the tissue specific toxicity of 6TG in vivo in transgenic Hprt-deficient mice. The approximate lethal dose for Hprt-deficient mice was 23-fold higher than for the wild-type. Serum biochemical analyses of 6TG-treated wild-type mice showed elevated serum enzyme levels characteristic of liver damage whereas the levels in Hprt-deficient 6TG-treated mice were within normal physiological limits. Histopathological examination of tissues from wild-type and from Hprt-deficient mice showed contrasting spectra of microscopic lesions. Wild-type mice had loss of hematopoietic cells from bone marrow starting at the lowest dose of 25 mg/kg 6TG whereas Hprt-deficient mice had normal bone marrow and spleen even at doses of 720 mg/kg 6TG. Wild-type mice also experienced severe loss of epithelial cells from the gastrointestinal tract starting at 50 mg/kg; however, the gastrointestinal tract of Hprt --/-- mice remained unaffected. Wild-type livers revealed atrophy and necrosis at doses of 25 mg/kg 6TG although Hprt --/-- livers displayed no effect until 507 mg/kg.

**Acute toxicity of 6TG.** As a quantitative parameter of 6TG toxicity we determined the approximate lethal dose that corresponds to the LD_{50} ± 30% for most chemicals (Deichmann and LeBlanc, 1943). The approximate lethal dose in wild-type 129/J mice was 50 mg/kg. The previously published LD_{50} value of 90 mg/kg was obtained in mice (Philips et al., 1954). Considering that different mouse strains were used...
our approximate lethal dose corresponds well with that value.

Acute toxicity of 6TG is delayed so that mice treated with the lowest lethal dose survived for 8 to 13 days, and even mice treated with 10 times the approximate lethal dose still survived for 4 days after administration (table 1). Delayed toxicity of 6TG has been found previously and has been proposed to result from agranulocytosis or thrombocytopenia and to resemble the toxic effects of ionizing radiation (Philips et al., 1954). It is likely that 6TG has to be metabolized and that the active 6TG metabolites cause damage in proliferating or metabolizing tissue. This may have caused animals death due to liver, hematopoietic and/or gastrointestinal failure. These effects are seen with most of the other purine analog chemotherapeutic agents (Philips et al., 1954).

**Tissue specific toxicities of 6TG.** Tissues from Hprt wild-type and Hprt mutant mice given a range of 6TG doses had different spectra of microscopic lesions. It has been previously shown that 6TG toxicity at fatal doses in wild-type mice is predominantly limited to the bone marrow (Philips et al., 1954). Our data show, that besides anticipated loss of hematopoietic cells, the 6TG treated wild-type mice also had loss of gastrointestinal epithelial cells and liver necrosis. Livers from 6TG-treated Hprt wild-type mice had atrophy, centrilobular hypertrophy and some liver cell necrosis. These lesions may be due to local effects of 6TG metabolites pro-

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**Fig. 2.** Microscopic lesions in the bone marrow of wildtype (Hprt +/+ ) mice treated with 100 mg/kg 6TG (A) compared to Hprt deficient (Hprt −/− ) mice administered 507 mg/kg of 6TG (B). A, Bone marrow of wildtype (Hprt +/+ ) mice treated with 100 mg/kg 6TG. Uniform, small round to discoid anuclear cells are mature erythrocytes (open arrowhead). They may have entered into vacant spaces and are not usually prominent in normal bone marrow. Vessels and fibrous connective stroma (solid arrowhead) are prominent due to decreased number of normal marrow elements. The very few remaining bone marrow hematopoietic precursor cells (open arrow) have indistinct or disrupted cytoplasm and/or pyknotic or fragmented nuclei diagnostic of necrosis. B, Bone marrow of Hprt-deficient (Hprt −/− ) mice administered 507 mg/kg of 6TG. The bone marrow from a treated Hprt-deficient mouse is within normal limits. Usual numbers and ratios of hematopoietic cells are present (open arrow, megakaryocytes; solid arrow, neutrophil cell lineage). Compared to wildtype, erythrocytes are not prominent. (Magnification 100×, staining hematoxilin and eosin.)

**Fig. 3.** A, Intestinal epithelium of wild-type (Hprt +/+ ) treated with 100 mg/kg 6TG. Representative section of intestine from a wild-type mouse with epithelial surface (solid arrow), submucosa (solid arrowhead) and muscle and serosal layers (open arrow). The epithelium has marked atrophy and necrosis characterized by decreased size and number of cells (open arrowhead). B, Intestinal epithelium of Hprt-deficient (Hprt −/− ) mice administered 720 mg/kg 6TG. Intestine from Hprt-deficient mouse has epithelium within normal limits. The epithelial surface (solid arrow) has normal size and numbers of cells as do the submucosa (solid arrowhead) and muscle layers (open arrow). (Magnification 100×, staining hematoxilin and eosin.)
duced in the hepatocytes. Observed liver damage correlated with serum biochemical analysis by increased levels of ASP and ALT (table 3A). It seems interesting that in rats and dogs damage to the bone marrow seems to be the primary cause of death after 6TG administration and that only slight if any damage to the liver is found (Philips et al., 1954). However, in man in agreement with our data myelosuppression is the primary complication of 6TG therapy and ornithinase muticosis and hepatitis were frequent secondary side effects (Bleyer, 1985).

Hprt deficiency in the transgenic mice caused marked protection for hematopoietic tissues and intestinal epithelium against 6TG toxicity. Hprt +/− mice given sublethal doses (720 mg/kg or less) of 6TG had normal bone marrow and gastrointestinal tract epithelium. However, after lethal doses (1148 mg/kg or higher) the animals displayed bone marrow lesions similar to treated wild-type mice (table 2). One possible explanation for this observation is activation of 6TG by an alternate metabolic pathway with lower specificity for 6TG. Although, Aprt is considered specific for adenine (Blakley, 1986) we cannot exclude that high doses of 6TG in these animals might compete with adenine for enzymatic conversion to 6TGMP. Cells deficient in Hprt activity frequently exhibit elevated Aprt activity (Brockman, 1974; Davidson and Winter, 1964). Thus, inhibition of Aprt enzymatic activity might decrease toxicity of 6TG in Hprt-deficient mice. It has been shown that Aprt activity is inhibited in mice by injection of 9-ethyladenine (Wu and Melton, 1993). Hprt-deficient mice do not show any symptoms of the human Lesch-Nyhan syndrome; however, Aprt inhibition in Hprt-deficient mice resulted in clinical manifestation of Lesch-Nyhan syndrome. However, our experiment indicates that 9-ethyladenine pretreatment did not protect Hprt-deficient animals against 6TG toxicity suggesting that Aprt is not responsible for the remaining toxicity of 6TG in Hprt-deficient mice. Although it has been shown that 9-ethyladenine inhibits Aprt activity in the brains of Hprt-deficient mice, no data on the inhibition of Aprt activity in the bone marrow and liver exist. Thus, as alternative explanation for our results we cannot completely rule out that Aprt was not inhibited by 9-ethyladenine in the target organs of 6-TG toxicity.

Renal lesions including evidence of tubule and glomerular damage seen only in Hprt −/− mice (table 2) may be associated with excretion of large amounts of 6TG catabolites such as 6-thiourea in the kidneys. Administration of adenine, purine or 2-chloroadenine causes the “adenine kidney” characterized by precipitation of crystals and induction of lesions (Philips et al., 1954). However, such crystals were not visible in our study or after administration of 6MP in mice or man (Philips et al., 1954). It is possible that the 6TG catabolites do not form visible crystals but still occur and cause irritation and lesions. Renal lesions were mainly developed at sublethal 6TG doses of 150 to 720 mg/kg in Hprt-deficient mice. Wild-type mice did not survive these doses. Hprt-deficient mice at lethal doses (1148 mg/kg or more) also had diminished renal lesions (table 2) indicating that biodegradation, excretion and/or time might be required for changes seen at sublethal doses. However, the 6TG catabolic products have not been characterized in Hprt-deficient animals. Thus, different catabolic products might be formed in Hprt-deficient mice that are more toxic to kidneys.

Implications for cancer treatment. There is some suggestive evidence that Hprt levels might be important for the efficacy of 6TG cancer treatment. Among 120 children with ALL, patients with lower incorporation of 6TG into 6TG nucleotides show a significant higher risk of relapse than patients with higher incorporation (Lennard and Lilleyman, 1989). Furthermore, among 83 children with untreated ALL, low Hprt activity is correlated with a poorer prognosis (Pieters et al., 1992). Similarly, among 44 children with untreated ALL, the probability of continuous complete remission was significantly lower in patients with 6TG-resistant cells (Pieters et al., 1991). Furthermore, patients with untreated chronic lymphocytic leukemia had significantly lower Hprt activities than control subjects and the Hprt activities were quite widely dispersed (Rambotti and Davis, 1981). However, other studies did not find a correlation between 6TG or 6MP resistance and HPRT activity (Davidson and Winter, 1964; Pieters et al., 1992). The underlying reason may be that a variety of other factors may contribute to drug resistance including lower affinity of Hprt for the ribose-phosphate donor 5′-phosphoribosyl-1-pyrophosphate, increased degradation of the drug, decreased incorporation of the analog into polynucleotides, and failure of the analog to enter the cells (Brockman, 1974).

However, there is quite some interindividual variation in 6TG resistance of cells of phenotypically normal individuals (Yamanaka et al., 1985). There is also considerable interindividual variability of the frequency of mutations giving rise to 6TG resistance (Davies et al., 1992) and normal, nontransformed cells from patients with cancer prone diseases such as Werners syndrome (Fukuchi et al., 1990). Ataxia telangiectas-

### Table 3

Biochemical analysis of mouse serum after treatment with 6TG

<table>
<thead>
<tr>
<th>6TG (mg/kg)</th>
<th>BUN (mg/dl)</th>
<th>AST (u/liter)</th>
<th>ALT (u/liter)</th>
<th>CK (u/liter)</th>
<th>AP (u/liter)</th>
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<tbody>
<tr>
<td>Physiol. range</td>
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<td>up to 75</td>
<td>up to 50</td>
<td>up to 50</td>
<td>up to 100</td>
</tr>
<tr>
<td>A) Hprt +/+</td>
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<td>45 ± 25</td>
<td>256 ± 74</td>
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<tr>
<td>B) Hprt −/−</td>
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<td></td>
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<td>20 ± 3</td>
<td>43 ± 14</td>
<td>14 ± 2</td>
<td>37 ± 11</td>
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</table>

Mice were treated i.p. with single doses of 6TG, control animals were treated with the same volume of sterile distilled water. The serum samples were taken 14 days after treatment and frozen until analysis. The values represent the averages ± S.D. from three animals. BUN, blood urea nitrogen; AST, aspartate amino transferase; ALT, alanine amino transferase; CK, creatinine kinase; AP, alkaline phosphatase.
tasia (Cole and Arlett, 1994), Bloom’s syndrome (Vijayalaxmi et al., 1983) and xeroderma pigmentosum (Cole et al., 1992) show a significantly elevated frequency of mutations to 6TG resistance. Thus, interindividual differences in 6TG sensitivity of lymphocytes (Lennard and Lilleyman, 1989) and of the frequency of mutations causing 6TG resistance may be responsible in part for the variability of the efficacy observed for 6TG chemotherapy.

Bone marrow cell hyperproliferation of surviving cells is commonly a result of toxicity to bone marrow cells (Jutter and To, 1992). Thus, if an increased fraction of 6TG-resistant bone marrow cells exist, killing of the 6TG-sensitive cells by 6TG might select for such 6TG-resistant cells. To avoid this, the sensitivity of lymphocytes to 6TG could be determined before and during administration of 6TG by lymphocyte cloning or other methods (Dempsey et al., 1983; Veerman and Pieters, 1990; Yamanaka et al., 1985).

The general approach of using transgenic animals with specific defects in drug metabolism pathways can contribute to the understanding of the mechanisms of toxicity and action of drugs. This could ultimately lead to improvement of drug design and treatment regimes.

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References


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