Br J clin Pharmac 1994; **38**: 1–8

Thiopurine methyltransferase activity in a French population: h.p.l.c. assay conditions and effects of drugs and inhibitors

E. JACQZ-AIGRAIN, E. BESSA, Y. MEDARD, Y. MIRCHEVA[2] & E. VILMER[1]
Services de Pharmacologie Clinique and [1]Immunohématologie (Hôpital Robert Debré), and [2]Unité INSERM U75, Paris, France

1 Thiopurine methyltransferase (TPMT) is a cytosolic enzyme involved in the catabolism of thiopurine drugs, which are used to treat cancer patients and organ transplant recipients. Because TPMT activity is polymorphic and under genetic control, large interindividual variations in the immunosuppressive activity and toxicity of these drugs may, at least in part, be inherited.

2 We have developed a specific h.p.l.c. method for measuring 6-methyl mercaptopurine formed from 6-mercaptopurine (6-MP) in red blood cell lysates during the TPMT assay procedure. In blinded assays of 55 samples from adult blood donors, the results of the h.p.l.c. method correlated with those of the radiochemical reference method ($r = 0.83$, $P < 0.001$).

3 Using this h.p.l.c. assay, we tested the effect of known inhibitors of TPMT activity (syringic acid, *p*-anisic acid and tropolone) *in vitro* and showed that they were highly inhibitory. We also found that drugs often administered concomitantly with 6-MP (prednisone, prednisolone, 6-methylprednisolone, cyclophosphamide, methotrexate, and trimethoprim-sulphamethoxazole) had little or no effect on TPMT activity *in vitro*.

4 In a group of 300 French individuals, TMPT activity was highly variable, ranging from 4.7 to 35.3 nmol h^{-1} ml^{-1} of packed red blood cells (nmol h^{-1} ml^{-1} PRBC) with a mean value of 19.3 ± 4.9. TMPT activity was not influenced by sex.

5 This sensitive and reproducible h.p.l.c. assay for TPMT activity in red blood cells may prove useful for prospective clinical studies designed to optimise dosage regimens of thiopurine drugs (detection limit for 6-methyl mercaptopurine is 5 ng ml^{-1}, intra- and inter-assay variations are 6.8 and 8.2%, respectively).

Keywords thiopurine methyltransferase genetic polymorphism

Introduction

Thiopurine methyltransferase (TPMT, EC 2.1.1.67) is a cytosolic enzyme involved in S-methylation of aromatic and heterocyclic sulphydryl compounds such as thiopurine and thiopyrimidine drugs [1,2]. The thiopurines azathioprine, 6-mercaptopurine and 6-thioguanine are used to treat cancer patients and organ transplant recipients.

In vivo, azathioprine is converted rapidly to 6-mercaptopurine (6-MP), which is metabolised by three competing pathways [3,4]. The anabolic pathway for 6-MP gives rise to active 6-thioguanine nucleotides (6-TGN), that are incorporated into DNA and RNA in cells with a nucleus [5–7]. The first catabolic pathway for 6-MP is oxidation to thiouric acid by xanthine oxidase (EC 1.2.3.2). The second catabolic pathway is methylation to 6-methylmercaptopurine. This reaction involves TPMT, the methyl group being provided by S-adenosyl-L-methionine (SAM) [8]. *In vitro*, erythrocyte TPMT activity was found to be negatively correlated with erythrocyte 6-TGN con-

Correspondence: Docteur Evelyne Jacqz-Aigrain, Service de Pharmacologie Clinique, Hôpital Robert Debré, 48, Boulevard Sérurier, 75019 Paris, France

centrations in children with acute lymphoblastic leukaemia taking 6-MP [9, 10]. As TPMT activity is a polymorphic reaction under genetic control [11,12–14], large interindividual variations in 6-MP immunosuppressive activity and toxicity may, at least in part, be inherited and involve differences in metabolism by the three pathways [9,12,15–18]. There are many examples of variations in drug responses due to genetic polymorphism of metabolism [19], and the determination of individual TPMT activity may thus be important for the optimisation of therapy.

The radiochemical (RC) assay is the reference method for the determination of TPMT activity in red blood cells [20]. It is based on the conversion of 6-mercaptopurine to radiolabelled 6-methylmercaptopurine (6-MMP), using radiolabelled SAM as the methyl donor. We modified this method by using cold SAM and measuring the amount of 6-MMP formed during the TPMT assay by means of h.p.l.c. after liquid-solid extraction. The h.p.l.c. method was compared with the RC method. We also investigated the potential inhibitory effects on TPMT activity of drugs given concomitantly with thiopurine derivates for the treatment of leukaemia. Finally, we used the h.p.l.c. assay to phenotype adult French blood donors.

Methods

Chemicals

6-mercaptopurine (6-MP) and dithiothreitol (DTT) were from Aldrich (Strasbourg, France). Allopurinol, 6-methylmercaptopurine (6-MMP), 3,5-dimethoxy-4-hydroxybenzoic acid (syringic acid), *p*-methoxybenzoic acid (*p*-anisic acid), 2-hydroxy-2-4-6 cycloheptatrienone (tropolone), prednisone, prednisolone, 6-methylprednisolone, cyclophosphamide, methotrexate and trimethoprim-sulphamethoxazole were from Sigma (St Louis, MO, USA). S-adenosyl-L-methionine hydrogen sulphate (SAM HSO_4, stable at + 4° C for several months) was from Boehringer (Mannheim, Germany). Chelex 100 was from Biorad (Richmond, CA, USA). All other chemicals were of analytical grade.

Metabolic assay of TPMT activity

Blood samples – preparation of lysates Samples were prepared as described previously [20]. Briefly, 5 ml of whole blood collected in lithium heparinized tubes was centrifuged at 800 *g* for 10 min at + 4° C to isolate red cells. After washing the pellet twice with 3 ml of normal saline and centrifugation for 10 min at 800 *g*, cells were gently resuspended in 2 ml of saline and the haematocrit was determined. After this step, the results of the assay can be expressed in terms of milliliters of packed red blood cells. Red cells were lysed with cold distilled water (4 ml for 1 ml of solution) and centrifuged (13,000 *g*, 10 min, + 4° C). The supernatant was kept at −80° C until analysis. TPMT is stable for several months under these conditions.

Incubation conditions Stock solutions of 6-MP in DMSO were kept at −20° C. Solutions of SAM, allopurinol and dithiothreitol in water were kept at −80° C for 1 month or less. After slow thawing, the erythrocyte lysate was chelated by adding 500 µl of Chelex-100 resin, followed by gentle rotation for 1 h at + 4° C and centrifugation at 4000 *g* for 10 min at + 4° C. Aliquots (200 µl) of the supernatant were placed in 5 ml glass tubes with potassium phosphate buffer (205 µl, 0.15 M, pH 7.5) and 6-MP (15 µl) and preincubated for 3 min at 37° C in a shaking water bath.

The reaction was started by adding 30 µl of a mixture containing SAM (final concentration: 10 µM), allopurinol, (an inhibitor of xanthine oxidase, final concentration: 20 µM), and DTT (to protect the thiopurine substrate from oxidation, final concentration: 250 µM). 6-MMP formation was measured initially in the presence of various concentrations of 6-MP (0.1–8 mM) and SAM (1–10 µM). For kinetic studies, the concentations of 6-MP were 0.1 to 8 mM for 6-MP and 10 µM for SAM. Standard incubation media contained 4 mM 6-MP and 10 µM SAM, in a final volume of 450 µl. The reaction was stopped after 1 h by adding 100 µl of hydrochloric acid (1 M).

In inhibition experiments, the inhibitor was added as a 10 µl solution (in water or DMSO). 6-MMP production was measured at 6-MP concentrations of 0.8 and 4 mM in control experiments (without inhibitor) and in incubation media containing 2 mM syringic acid, *p*-anisic acid or tropolone. Prednisone, prednisolone, 6-methylprednisolone, cyclophosphamide, methotrexate and trimethoprim-sulphamethoxazole were tested at a final concentration of 2 mM. The results of inhibition experiments are expressed as percentage TPMT activity relative to controls.

H.p.l.c. 6-MMP assay Standard curves of 6-MMP (25 to 250 ng ml^{-1}) were prepared from appropriate dilutions of the stock solution.

Liquid-solid extraction was performed on a polymeric MP1 column (Interaction Chemicals, Mountain View, CA, USA). Briefly, the column was activated by washing with 2 ml methanol and 4 ml phosphate buffer saline (PBS). After adding the sample, the column was washed with 2 ml PBS 0.01 m, pH 7.2, dried for 5 min and eluted with 2 ml methanol. The eluate was evaporated under air and the dry extract was dissolved in 200 µl of mobile phase, filtered and centrifuged. An aliquot (50 µl) was injected into the h.p.l.c. system.

The h.p.l.c. system consisted of a Spectra Physics model 8800 pump (SP, Santa Clara, CA, USA), an SP 8780 autosampler and an SP 8450 u.v. detector (280 nm). We used a C18/5-µm particle size Supelcosil LC (250 mm) column with a C18/5 µm LC precolumn (20 mm). The mobile phase was a mixture of acetonitrile and water containing 0.2%, w/v, acetic acid and 0.1%, w/v DTT (1 M). The flow rate was 1.0 ml min^{-1}, with gradients of 7:93, 80:20 and 7:93 (v/v) for 18, 3 and 15 min, respectively.

Radiochemical 6-MMP assay The radiochemical assay of TPMT activity in red blood cells was per-

formed, as described previously, on red blood cell lysates. Briefly, the assay is based on the conversion of 6-mercaptopurine to radiolabelled 6-methylmercaptopurine using radiolabelled S-adenosyl-L-methionine as the methyl donor [20].

Statistical analysis

TPMT activity is expressed in nanomoles of 6-MMP formed per milliliter of packed red blood cells per hour (nmol h^{-1} ml^{-1} PRBC).

All data points for the *in vitro* assays represent the results of at least two independent experiments. The apparent Michaelis-Menten constants [maximum velocity (V_{max}) and affinity constant (K_m)] were determined graphically.

Results are expressed as means ± standard deviation (mean ± s.d.). Comparisons between groups were made by using Student's *t*-test, with a threshold of significance of $P = 0.05$.

Results

H.p.l.c. 6-MMP assay

Under the chromatographic conditions described, the retention time of 6-MMP was 16.2 min. The recoveries of 25 and 100 μg ml^{-1} 6-MMP added to red blood cell lysates were 87.5 and 73.5%, respectively (n = 5). Chromatographic separation of 6-MMP is illustrated in Figures 1a (25 ng ml^{-1} 6-MMP) and 1b (100 ng ml^{-1} 6-MMP).

The calibration plots of peak areas *vs* concentrations were linear over the range of 6-MMP concentrations tested (25 to 250 ng ml^{-1}). The detection limit was 12.5 ng ml^{-1}, defined as half the concentration of the lowest concentration standard at a signal-to-noise ratio of 10:1.

The inter-assay coefficients of variation for assay of 25 and 100 ng ml^{-1} 6-MMP (n = 5) were 6.2 and 6.2%, respectively. The inter-assay CV for the slope of the calibration curve was 8.9% (n = 5).

Selection of appropriate blanks 6-MMP is formed in red blood cells in the presence of 6-mercaptopurine and S-adenosine-L-methionine (Figure 2b). However, 6-MMP was also detected when the lysate was incubated with 6-MP but without SAM (Figure 2a). The amount of 6-MMP measured in the SAM-free blanks increased linearly with the 6-MP concentration at fixed concentrations of lysate, but did not exceed 20% of the value obtained in the presence of

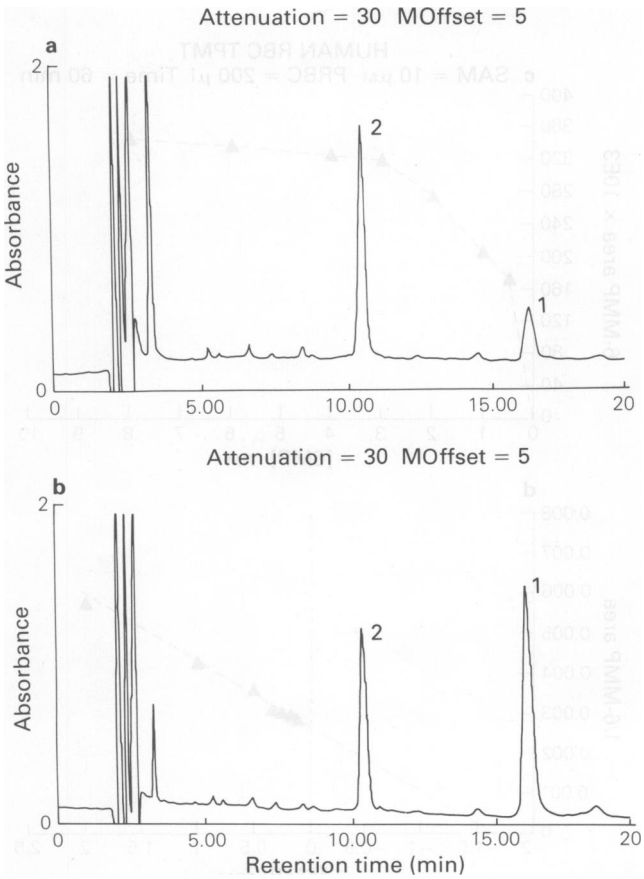

Figure 1 Chromatographic separation of 6-MMP. Peaks: 1 = 6-MMP, 2 = diethiothreitol. a) standard concentration of 25 ng ml^{-1}; b) standard concentration of 100 ng ml^{-1}.

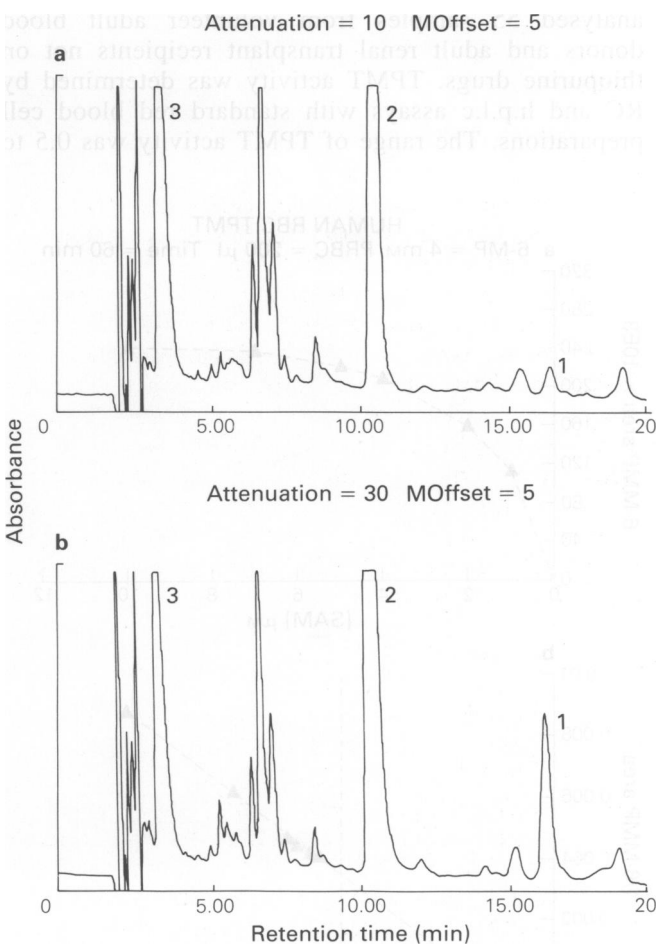

Figure 2 Chromatographic separation of 6-MMP Peaks: 1 = 6-MMP, 2 = diethiothreitol, 3 = 6-MP. The upper trace (a) represents the RBC blank (without SAM). The lower trace (b) represents the corresponding RBC sample (containing SAM). In this sample, 6-MMP formed was 50 ng ml^{-1}, and the corresponding TPMT activity was 15.4 nmol h^{-1} ml^{-1} PRBC.

SAM. In contrast, no 6-MMP was detected in buffer blanks containing 6-MP and SAM but no lysate. We thus used blanks including all the assay reagents except the co-substrate and each sample served as its own blank.

TPMT activity Intra-assay variation, determined by measuring TPMT activity five times in a single run, was 6.8%

Inter-assay variation, determined by measuring TPMT activity five times in different runs, was 8.2%.

TPMT activity fell by 7% in samples (n = 5) kept for 3 months at −20° C and by 15% in samples (n = 5) kept for sixteen months at −80° C.

Kinetics of TPMT activity in red blood cell lysates 6-MMP formation was linear with respect to the lysate concentration (50–200 µl/assay) and time (15–60 min) (Figure 3). 6-MMP formation was measured in the presence of various concentrations of 6-MP (0.1–8 mM) and SAM (1–10 µM). The apparent K_m was 0.6 mM for 6-MP and 1.3 µM for SAM (Figure 4). The incubation conditions were 4 mM 6-MP and 10 µM SAM.

Comparison of the h.p.l.c. and RC methods We analysed 55 samples from volunteer adult blood donors and adult renal transplant recipients not on thiopurine drugs. TPMT activity was determined by RC and h.p.l.c assays with standard red blood cell preparations. The range of TPMT activity was 0.5 to

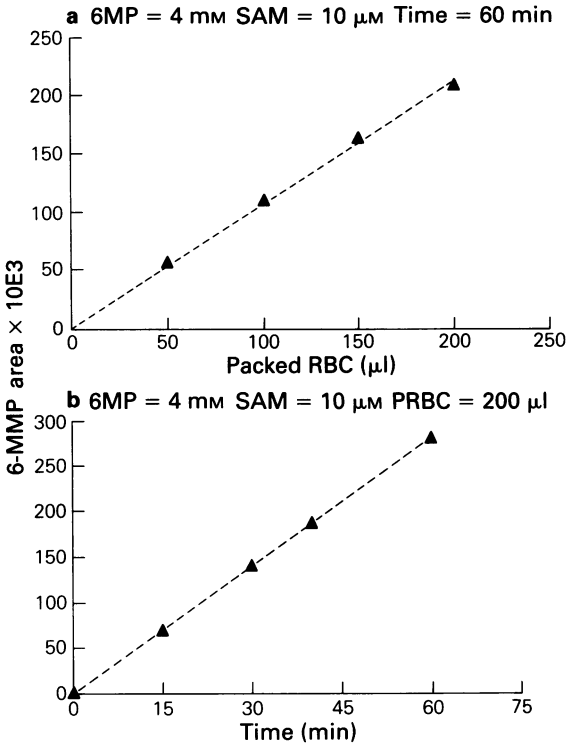

Figure 3 Effect of increasing quantities of erythrocyte lysate (a) and incubation time (b) on TPMT activity. Each point is the mean of 3 determinations.

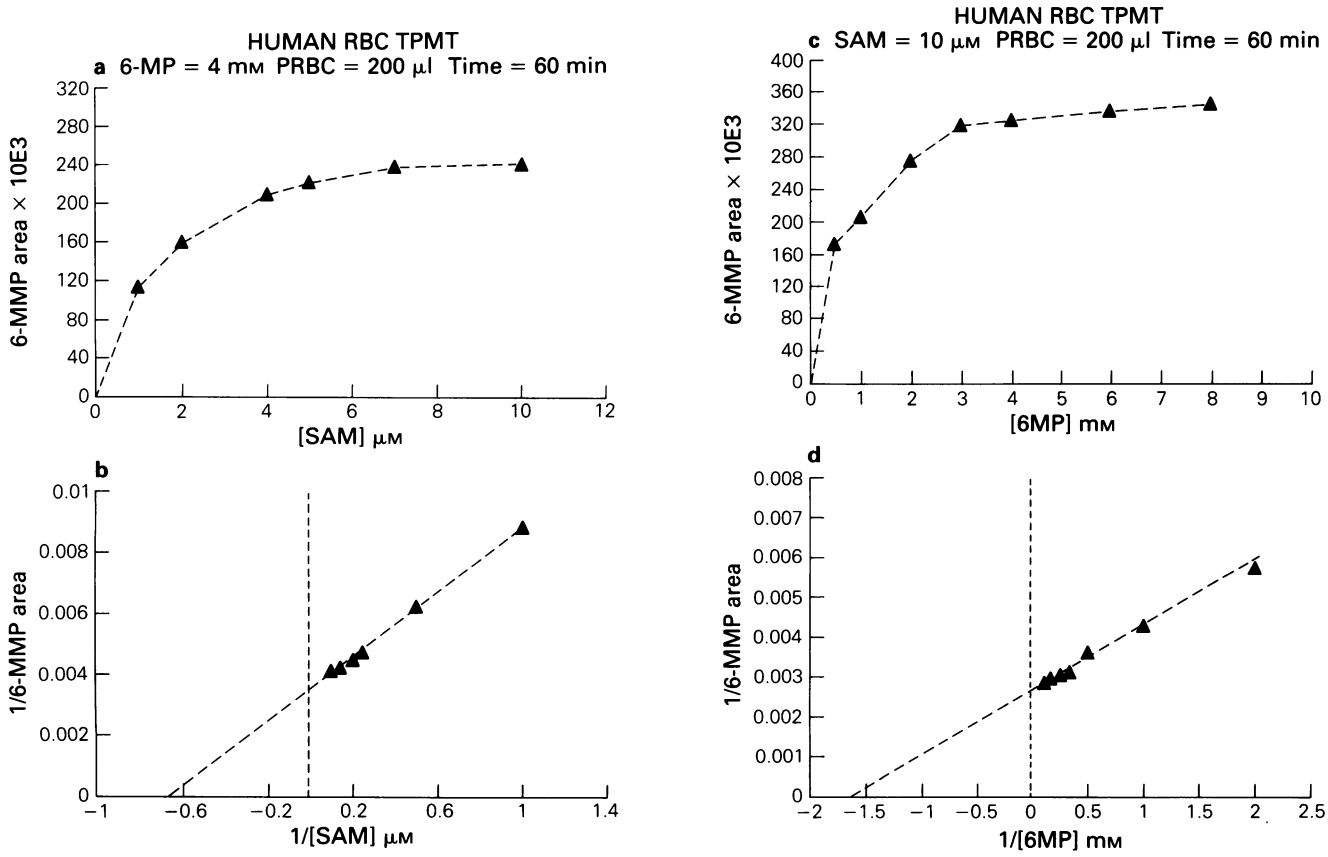

Figure 4 Effects of substrate and co-substrate concentrations on TPMT activity: kinetic data.
RBC TPMT activity was determined in the presence of increasing concentrations of SAM (a: substrate curve, b: double reciprocal plot) and a 6-MP concentration of 4 mM, and in the presence of increasing concentrations of 6-MP (c: substrate curve, d: double reciprocal plot) at a SAM concentration of 10 µM. Each point is the mean of 3 determinations.

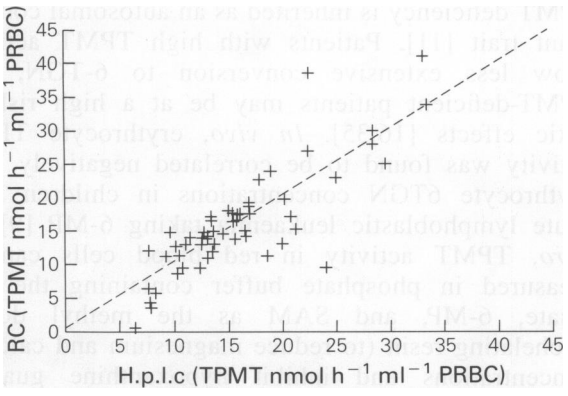

Figure 5 Linear regression of TPMT values measured by the h.p.l.c. and RC assays. TMPT activity is expressed as the amount of 6-MMP (nmol) formed per hour and per ml of PRBC. y = 1.016, x –0.083 ($r^2 = 0.69$, $P < 0.0001$).

33.7 nmol h^{-1} ml^{-1} PRBC by the RC method and 4.6 to 35.3 nmol h^{-1} ml^{-1} PRBC by the h.p.l.c. method. The mean TPMT activities obtained by the h.p.l.c. and RC methods were 15.4 ± 6.9 and 15.6 ± 8.2, respectively ($P > 0.05$).

Linear regression analysis of h.p.l.c. *vs* RC TPMT values yielded the following relationship: (RC) TPMT = 1.016 (h.p.l.c.) TPMT–0.083, i.e. a slope near unity and a negligable intercept value. The Pearson product moment correlation coefficient (r^2) was 0.69 ($P = 0.0001$, Figure 5).

The difference between the two values (h.p.l.c.–RC) was calculated for each sample, and gave a mean of [–0.18 ± 4.29]. These differences were plotted against the mean of the two values [(h.p.l.c. + RC)/2] and the regression analysis yielded the following relationship: (h.p.l.c.–RC) = [–0.22 (h.p.l.c. + RC)/2] + 3.29. The slope was near zero and the intercept value was negligible.

The mean percentage difference (% Diff) between the two methods [(h.p.l.c. – RC)/RC] was 27% ± 161%.

Effects of inhibitors and drugs

Three known inhibitors of TPMT activity were tested. Syringic acid was highly inhibitory (residual TPMT activity < 1%), whereas residual activity was 30% with tropolone and *p*-anisic acid (Figure 6).

All six drugs tested had little or no effect on 6-MMP formation (residual TPMT activity was always more than 70% of control activity (Figure 6).

Values in a randomly selected French Caucasian population sample

TPMT activity was determind in 300 adult volunteers. The mean value was 19.3 ± 4.9 nmol h^{-1} ml^{-1} PRBC. No sex-related difference was observed (18.9 ± 4.9 nmol h^{-1} ml^{-1} PRBC in 166 females and 19.6 ± 4.9 nmol h^{-1} ml^{-1} PRBC in 134 males). The frequency distribution histogram is presented in Figure 7. Because of the relatively small number of subjects tested, the frequency distribution of

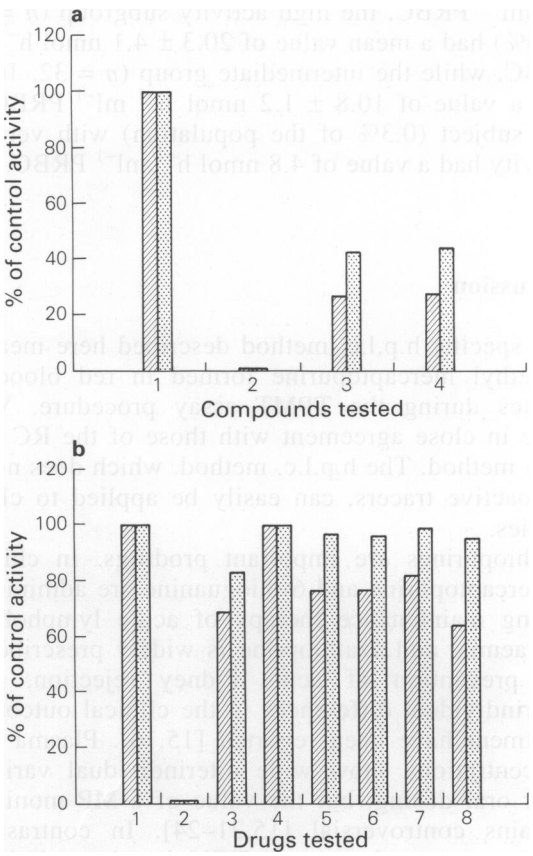

Figure 6 Inhibition of TPMT activity by various compounds (6-MP 0.8 and 4 mM, inhibitors 2 mM).
a) From left to right: control (1), syringic acid (2), *p*-methoxybenzoic acid (3) and tropolone (4).
b) From left to right: control (1), syringic acid (2), prednisone (3), prednisolone (4), 6-methylprednisolone (5), cyclophosphamide (6), methotrexate (7), trimethoprimsulphamethoxazole (8).
Results are expressed as the percentage (%) of the control value (no inhibitor). ▨ 6-MP 0.8 mM; ▩ 6-MP 4 mM.

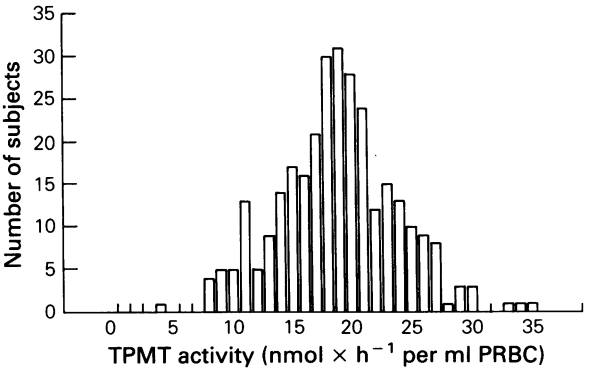

Figure 7 Frequency distribution histogram of TPMT activity in 300 French Caucasians.

TPMT activity appeared to be gaussian (Skewness coefficient: 0.11; Kurtosis coefficient: 0.26).

However, activity varied widely, from 4.8 to 35.3 nmol h^{-1} ml^{-1} PRBC. Approximately 90% of the subjects had relatively high TPMT activity, while 10% had intermediate activity and one had very low activity. With a cut-off between the high and intermediate activity subgroups set arbitrarily at 12 nmol

h^{-1} ml^{-1} PRBC, the high activity subgroup (n = 267, 89.0%) had a mean value of 20.3 ± 4.1 nmol h^{-1} ml^{-1} PRBC, while the intermediate group (n = 32, 10.7%) had a value of 10.8 ± 1.2 nmol h^{-1} ml^{-1} PRBC; the one subject (0.3% of the population) with very low activity had a value of 4.8 nmol h^{-1} ml^{-1} PRBC.

Discussion

The specific h.p.l.c. method described here measures 6-methyl mercaptopurine formed in red blood cell lysates during the TPMT assay procedure. Values were in close agreement with those of the RC reference method. The h.p.l.c. method, which does not use radioactive tracers, can easily be applied to clinical studies.

Thiopurines are important prodrugs. In children, 6-mercaptopurine and 6-thioguanine are administered during maintenance therapy of acute lymphoblastic leukaemia, and azathioprine is widely prescribed for the prevention of acute kidney rejection. Large interindividual differences in the clinical outcome of treatment have been reported [15,16]. Plasma 6-MP concentrations show wide interindividual variations after oral dosing, but the value of 6-MP monitoring remains controversial [15,21–24]. In contrast, the RBC content of active 6-TGN has been linked to cytotoxicity and prognosis in children receiving 6-MP for acute lymphoblastoic leukaemia [9,12,15–18]. In addition, sex-related differences in safety and outcome have been noted in children with lymphoblastic leukaemia [25–27].

The metabolism of 6-mercatopurine involves three competing pathways. The initial activation step of the anabolic pathway is catalysed by the enzyme hypoxanthine phosphoribosyltransferase (HPRT, EC 2.4.2.8). There is little variation in RBC HPRT activity, as there was a 1.7 fold range in a control population of 86 children and a 1.3 fold range in 63 children with acute lymphoblastic leukaemia, with higher activity in boys than in girls [28]. The first catabolic pathway for 6-MP is oxidation to thiouric acid by xanthine oxidase (EC 1.2.3.2). There is little interindividual variation in the activity of this enzyme when measured in healthy subjects with the caffeine as probe [29,30]. In contrast, sex-related differences have been detected in liver tissue *in vitro*, with higher activity in men than in women [31,32]. The second catabolic pathway is S-methylation to 6-methylmercaptopurine. This reaction involves TPMT, the methyl group being provided by S-adenosyl-L-methionine (SAM) [8]. TPMT is a cytosolic enzyme widely distributed in red blood cells, lymphocytes, platelets, and kidney and liver cells [13,14,33]. TPMT can catalyse the methylation of aromatic thiol compounds, including thiopurine and thiopyrimidine drugs. This activity should be referred to as 'aryl thiol methyltransferase' and is now clearly distinguished from membrane-bound thiol methyltransferase activity (EC 2.1.1.9) [34]. Population studies have shown a trimodal distribution of TPMT activity [12–14] and family studies have demonstrated that TPMT deficiency is inherited as an autosomal codominant trait [11]. Patients with high TPMT activity show less extensive conversion to 6-TGN, and TPMT-deficient patients may be at a high risk of toxic effects [16,35]. *In vivo*, erythrocyte TPMT activity was found to be correlated negatively with erythrocyte 6TGN concentrations in children with acute lymphoblastic leukaemia taking 6-MP [9]. *In vitro*, TPMT activity in red blood cells can be measured in phosphate buffer containing the cell lysate, 6-MP, and SAM as the methyl donor. A chelating resin (to reduce magnesium and calcium concentrations and inhibit hypoxanthine guanine phosphoribosyltransferase) and allopurinol (which inhibits xanthine oxidase) are also added. The RC TPMT assay is based on the conversion of 6-mercaptopurine to radiolabelled 6-methylmercaptopurine using radiolabelled SAM ([^{3}H] or [^{14}C] with essentially similar results [20,36]. Radioactivity was detected in experiments with substrate-free blank, due to radioactively labelled methanol formed in the presence of SAM. In contrast, the h.p.l.c. assay is based on the conversion of 6-mercaptopurine to 6-methylmercaptopurine and uses cold SAM. 6-MMP was detected in experiments with SAM-free blanks, but values never exceeded 20% of those observed in incubation media containing exogenous SAM. This 6-MMP formation may be due to the presence of endogeneous SAM in the red blood cell lysates. We did not observe non-enzymatic production of 6-MMP in buffer blanks. Recovery after extraction was better than 70%, and reproducibility and repeatability were satisfactory. The apparent Michaelis-Menten constants for 6-MP and SAM were similar to those reported previously with the RC method: K_m values for 6-MP were 3.2×10^{-4} M and 6×10^{4} M in the RC and h.p.l.c. method, respectively, and 1.7×10^{-6} M and 1.3×10^{-6} M for SAM. The h.p.l.c. values were in close agreement with those of the RC method [37] and h.p.l.c. can be used for the routine determination of TPMT activity in red blood cells.

Using the h.p.l.c. method, we measured TPMT activity in the presence of the inhibitors syringic acid, *p*-methoxybenzoic acid and tropolone [38], and showed that the activity measured is indeed dependent on TPMT.

Six drugs commonly used concomitantly with thiopurine derivates in the treatment of acute lymphoblastic leukaemia had no influence *in vitro*. If the drug concentration *in vivo* is of the same order as or lower than the high concentration we used *in vitro*, these drugs should have no influence on TPMT activity when combined with a thiopurine.

TPMT activity was determined initially in a population sample of 298 Caucasian Americans [11], then in adults Caucasians from France [36] and Norway [39], and in British children [9] (Table 1). In our French Caucasian population sample, the range of individual TPMT activity was wide (4.8 to 35.3 nmol h^{-1} ml^{-1} RBC) and similar to reported values, and we distinguished three groups of subjects with high, intermediate and very low TPMT activity. However, mean TPMT activity appeared to be higher than the

Table 1 Inter-ethnic differences in thiopurine methyltransferase activity (nmol h^{-1} ml^{-1} PRBC). Data are presented as mean and range

Population	*Number of subjects*	*Mean (median)*	*Range*	*Author and reference*
North American Caucasians	298	12.8	0–19	Weinshilboum [11]
French Caucasians	303	15.4	2–40	Tinel [36]
Norwegian Caucasians	50	13.1	6.7–19.7	Klemetsdal [39]
Norwegian Saami	123	(15.9)	0.3–37.0	Klemetsdal [42]
Chinese	119	not stated	10–44	Lee [41]
North American Blacks	115	8.6	2.0–16.6	Jones [40]
French Caucasians	300	19.3	4.7–35.3	Present study

mean values previously reported in Caucasians. The difference between studies might be due to random selection of a small number of subjects to characterize populations for this highly variable character, in which case it would be reduced by increasing the number of subjects. TPMT activity has also been measured in other ethnic groups namely, North American Blacks [40], Chinese [41] and Saami [42]. A gender difference was detected only in the Norwegian population, men having higher TPMT activity [42].

Thiopurine-induced myelotoxicity is clearly multifactorial [43] but may be associated with very low or undetectable TPMT activity [16,35,44–46]. The determination of TPMT activity may thus be of importance in preventing severe thiopurine toxicity in patients lacking TPMT activity, but also in optimising dosage regimens in patients with high or intermediate activity. Our h.p.l.c. assay, which does not involve the use of radioactive reagents, could easily be used by a hospital laboratory for clinical studies.

References

1 Ames MM, Selassie CD, Woodson LC, Van Loon JA, Hansch C, Weinschilboum RM. Thiopurine methyltransferase: Structure-activity relationships for benzoic acid inhibitors and thiophenol substrates. *J med Chem* 1986; **29**: 354–358.

2 Remy CN. Metabolism of thiopyrimidines and thiopurines S-methylation with S-adenosyl-methionine transmethylase and catabolism in mammalian tissues. *J biol Chem* 1963; **238**: 1078–1084.

3 Lennard L. The clinical pharmacology of 6-mercaptopurine. *Eur J clin Pharmac* 1992; **43**: 329–339.

4 Bostrom B, Erdmann G. Cellular pharmacology of 6-mercaptopurine in acute lymphoblastic leukaemia. *Am J Ped Hematol/Oncol* 1993; **15**: 80–86.

5 Lennard L, Maddocks JL. Assay of 6-thioguanine nucleotide, a major metabolite of azathioprine, 6-mercaptopurine and 6-thioguanine, in human red blood cells. *J Pharm Pharmac* 1983; **35**: 15–18.

6 Lennard L. Assay of 6-thioinosinic acid and 6-thioguanine nucleotides, active metabolites of 6-mercaptopurine, in human red blood cells. *J Chromatogr* 1987; **432**: 169–178.

7 Tidd DM, Paterson ARP. A biochemical mechanism for the delayed cytotoxic reaction of 6-mercaptopurine. *Cancer Research* 1974; **34**: 738–746.

8 Remy CN. Ribonucleotides and ribonucleosides as methyl acceptors for SAM. *Biochem Biophys Acta* 1967; **138**: 258–275.

9 Lennard L, Lilleyman JS, Van Loon J, Weinshilboum RM. Genetic variation in response to childhood acute lymphoblastic leukaemia. *Lancet* 1990; **ii**: 225–229.

10 Lennard L, Van Loon JA, Lilleyman JS, Weinshilboum RM. Thiopurine pharmacogenetics in leukemia: correlation of erythrocyte thiopurine methyltransferase activity and 6-thioguanine nucleotides. *Clin Pharmac Ther* 1987; **41**: 18–25.

11 Weinshilboum RM, Sladek SL. Mercaptopurine pharmacogenetics: Monogenic inheritance of erythrocyte thiopurine methyltransferase activity. *Am J Hum Genet* 1980; **32**: 651–662.

12 Weinshilboum RM. Pharmacogenetics of methyl conjugaison and thiopurine drug toxicity. *Bio-assays* 1987; **7**: 78–82.

13 Woodson LC, Dunnette JH, Weinshilboum RM. Pharmacogenetics of human thiopurine methyltransferase: Kidney erythrocyte correlation and immunotitration studies. *J Pharmac exp Ther* 1982; **222**: 174–181.

14 Van Loon JA, Weinshilboum RM. Thiopurine methyltransferase biochemical genetics: human lymphocyte activity. *Biochem Genet* 1982; **20**: 637–638.

15 Lennard L, Lilleyman JS. Variable 6-mercaptopurine metabolism and treatment outcome in childhood lymphoblastic leukemia. *J clin Oncol* 1989; **7**: 1816–1823.

16 Lennard L, Van Loon JA, Weinshilboum RM. Pharmacogenetics of acute azathioprine toxicity: relationship to thiopurine methyltransferase genetic polymorphism. *Clin Pharmac Ther* 1989; **46**: 149–154.

17 Lennard L, Lilleyman JS. Are children with lymphoblastic leukaemia given enough 6-mercaptopurine? *Lancet* 1987; **ii**: 785–787.

18 Van Loon JA, Weinshilboum RM. Human lymphocyte thiopurine methyltransferase pharmacogenetics: Effect of phenotype on 6-mercaptopurine induced inhibition of mitogen stimulation. *J Pharmac exp Ther* 1987; **242**: 21–26.

19 Jacqz E, Hall SD, Branch RA. Genetically determined polymorphisms in drug oxidation. *Hepatology* 1986; **6**: 1020–1032.

20 Weinshilboum RM, Raymond FA, Pazmino PA. Human erythrocyte thiopurine methyltransferase: Radiochemical microassay and biochemical properties. *Clin Chim Acta* 1978; **85**: 323–333.

21 Koren G, Ferrazini G, Sulh H, Langevin AM, Kapelushnik J, Klein J, Giesbrecht E, Soldin S, Greenberg M. Systemic exposure to mercaptopurine as a prognostic factor in acute lymphocystic leukemia in children. *New Engl J Med* 1990; **323**: 17–21.

22 Hayder S, Lafolie P, Bjork O, Peterson C. 6-mercapto-

purine plasma levels in children with acute lymphoblastic leukemia: relation to relapse risk and myelotoxicity. *Ther Drug Monit* 1989; **11**: 617–622.
23 Rundles RW, Elion GB. 6 MP bioavailability. *New Engl J Med* 1984; **310**: 929–932.
24 Zimm S, Collins JM, Riccardi R, O'Neill D, Narang PK, Chabner B, Poplack DG. Variable bioavailability of oral 6-mercaptopurine. *New Engl J Med* 1983; **308**: 1005–1009.
25 Lilleyman JS, Lennard L, Rees CA, Morgan G, Maddocks JL. Childhood lymphoblastic leukaemia: sex difference in 6-mercaptopurine utilization. *Br J Cancer* 1984; **49**: 703–707.
26 Hale JP, Lilleyman JS. Importance of 6-mercaptopurine dose in lymphoblastic leukemia. *Arch Dis Child* 1991; **66**: 462–466.
27 Sather M, Miller D, Nesbit M, Heyn R, Hammond D. Difference in prognosis for boys and girls with acute lymphoblastic leukemia. *Lancet* 1981: **i**: 739–743.
28 Lennard L, Hale JP, Lilleyman JS. Red blood cell hypoxanthine phosphoribosyltransferase activity measured using 6-mercaptopurine as a substrate: a population study in children with acute lymphoblastic leukaemia. *Br J clin Pharmac* 1993; **36**: 277–284.
29 Grant DM, Tang BK, Kalow W. Variability in caffeine metabolism. *Clin Pharmac Ther* 1983; **33**: 591–602.
30 Grant DM, Tang BK, Kalow, W. Effect of allopurinol on cafeine disposition in man. *Br J clin Pharmac* 1986; **21**: 454–458.
31 Guerciolini R, Szumlanski C, Weinshilboum RM. Human liver xanthine oxidase: nature and extent of individual variation. *Clin Pharmac Ther* 1991; **50**: 663–672.
32 Relling MV, Lin JS, Ayers GD, Evans WE. Racial and gender differences in N-acetyltransferase, xanthine oxidase, and CYP1A2 activities. *Clin Pharmac Ther* 1992; **52**: 648–658.
33 Szumlanski CL, Honchel R, Scott MC, Weinshilboum RM. Human liver thiopurine methyltransferase pharmacogenetics: biochemical properties, liver-erythrocyte correlation and presence of isozymes. *Pharmacogenetics* 1992; **2**: 148–159.
34 Woodson LC, Ames MM, Selassie CD, Hansch C, Weinshilboum RM. Thiopurine methyltransferase: Aromatic thiol substrates and inhibition by benzoic acid derivates. *Mol Pharmac* 1983; **24**: 471–478.
35 Evans WE, Horner M, Qin Chu Y, Kalvinsky D, Roberts WM. Altered mercaptopurine metabolism, toxic effects and dosage requirement in a thiopurine methyltransferase-deficient child with acute lymphoblastic leukemia. *J Pediatr* 1991; **119**: 985–989.
36 Tinel M, Berson A, Pessayre D, Letteron P, Cattoni MP, Horsmans Y, Larrey D. Pharmacogenetics of human erythrocyte thiopurine methyltransferase activity in a French population. *Br J clin Pharmac* 1991; **32**: 729–734.
37 Bland JM, Altman D. Statistical method for assessing agreement between two methods of clinical measurement. *Lancet* 1986; **i**: 307–310.
38 Woodson LC, Weinshilboum RM. Human kidney thiopurine methyltransferase: purification and biochemical properties. *Biochem Pharmac* 1983; **32**: 819–826.
39 Klemetsdal B, Tollefsen E, Loennechen T, Johnsen K, Utsi E, Gisholt K, Wist E, Aarbakke J. Interethnic difference in thiopurine methyltransferase activity. *Clin Pharmac Ther* 1992; **51**: 24–31.
40 Jones CD, Smart C, Titus A, Blyden G, Dorvil M, Nwadike N. Thiopurine methyltransferase activity in a sample population of black subjects in Florida. *Clin Pharmac Ther* 1993; **53**: 348– 353.
41 Lee EJD, Kalow W. Thiopurine S-methyltransferase activity in a Chinese population. *Clin Pharmac Ther* 1993; **54**: 28–33.
42 Klemetsdal B, Straume B, Wist E, Aarbakke J. Identification of factors regulating thiopurine methyltransferase activity in a Norwegian population. *Eur J clin Pharmac* 1993; **44**: 147–152.
43 Soria-Royer C, Legendre C, Mircheva J, Premel S, Beaune P, Kreis H. Thiopurine methyltransferase activity to assess azathioprine myelotoxicity in renal transplant recipients. *Lancet* 1993; **341**: 1593.
44 McLeod HL. Azathioprine-induced myelosuppression in thiopurine methyltransferase deficient heart transplant recipient. *Lancet* 1993; **i**: 1151.
45 Schutz E, Gummert J, Mohr F, Oellerich M. Azathioprine-induced myelosuppression in thiopurine methyltransferase deficient heart transplant recipient. *Lancet* 1993; **341**: 436.
46 Chocair PR, Duley JA, Simmonds HA, Cameron JS. The importance of thiopurine methyltransferase activity for the use of azathioprine in transplant recipients. *Transplantation* 1992; **53**: 1051–1056.

(Received 12 July 1993, accepted 28 February 1994)