6-Mercaptopurine Pharmacokinetics After Use of Azathioprine in Renal Transplant Recipients with Intermediate or High Thiopurine Methyl Transferase Activity Phenotype

A. ESCOUSSE, F. GUEDON, J. MOUNIE, G. RIFLE*, C. MOUSSON* AND P. D'ATHIS†

*Biochimie Pharmacologique, *Néphrologie, †Informatique Médicale, C.H.U.-21033 Dijon Cedex, France*

Abstract

Prevention of allograft transplant rejection by the immunosuppressive 6-thiopurine drug azathioprine is limited by haematological toxicity (leucopenia or agranulocytosis). This toxicity is particularly apparent in subjects with low thiopurine methyltransferase activity (TPMTase) phenotype (1% in the Caucasian population). The thiopurine derivative 6-mercaptopurine is the active metabolite of azathioprine, and it would be of interest to measure, after validation of plasma measurements, the mean values of the pharmacokinetic parameters in transplant patients with high or intermediate TPMTase phenotypes (85 and 14% of the Caucasian population, respectively).

We measured erythrocyte TPMTase activity in 103 kidney transplant recipients of high or intermediate phenotype and calculated, after a test dose of azathioprine, the mean values of the pharmacokinetic parameters for 6-mercaptopurine. We also compared these values with the same parameters from one subject with low TPMTase activity phenotype. The mean observed area under the plasma concentration–time curve (AUC) was 190 ± 140 ng mL^{-1} h and the elimination rate constant (K_{el}) was 1.92 ± 1.

The pharmacokinetic parameters (AUC, K_{el}, t^{1}_{2el} (the elimination half-life)) of 6-mercaptopurine in transplant patients are normally distributed and suitable for acceptance as a gold standard value for this population of Caucasian transplant patients. It seems useful to calculate these parameters, representative of the systemic exposure of individual patients to the drug, before prescribing these subjects azathioprine immunosuppressive treatment. In subjects with low TPMTase phenotype these pharmacokinetic measurements could also be an index of dose reduction.

Over the past 25 years azathioprine has been one of the drugs most frequently used in combination therapy for prevention of allograft immune rejection after organ transplantation (Schwartz et al 1959). Currently, determination of therapeutic doses of azathioprine for prevention of allograft rejection remains empirical, and dose reduction is only recommended if the leukocyte count becomes too low.

Because azathioprine is a prodrug for 6-mercaptopurine, it can rapidly and spontaneously be converted into 6-mercaptopurine and 5-methylnitroimidazole, either by nucleophilic attack or enzymatically by glutathione-S-transferase. The overall immunosuppressive actions of azathioprine and 6-mercaptopurine are almost identical, but azathioprine has a better therapeutic index, resulting in

Correspondence: A. Escousse, Department of Pharmacology, Faculty of Medicine, 7 boulevard Jeanne d'Arc, 21000 Dijon, France.

less haematological toxicity for the same immunosuppressive effect (Elion 1967). This difference might be because of the chemosensitizing effect of the 5-substituted 1-methyl-4-nitro-5-thioimidazole on lymphocytes (Sauer et al 1988).

The active immunosuppressive (and myelotoxic) products are the intracellular metabolites of 6-mercaptopurine, 6-thioinosinic and 6-thioguanine nucleotides, but the exact mechanism of the immunosuppressive action is controversial (Brooks et al 1982; Chan et al 1987). The final metabolite of 6-mercaptopurine resulting from the degradation of these 6-thionucleotides is 6-thiouric acid. Allopurinol is a potent antagonist of xanthine oxidase activity, increasing the intracellular accumulation of these toxic nucleotides and inducing the haematological toxicity of 6-thiopurines (Van Scoik et al 1985).

In subjects treated with azathioprine (or 6-mercaptopurine), low enzymatic 6-thiopurine methyl-

transferase activity, genetically controlled (monogenic trait), is related to haematological toxicity, resulting also from the intracellular accumulation of the toxic 6-thionucleotides (Lennart et al 1989, 1990). The low thiopurine methyltransferase activity (TPMTase) phenotype is encountered in 1% of the Caucasian population. If this haematological toxicity is infrequent in the high TPMTase activity phenotype, it is unknown if the intermediate TPMTase phenotype (14% of the Caucasian population) is also linked to the much greater toxic effect of azathioprine.

The therapeutic efficiency of other 6-thiopurine drugs used as cytotoxics (6-mercaptopurine and 6-thioguanine) is dose-dependent and can be correlated with systemic exposure to the drug, as is shown by the modifications in the area under the plasma concentration–time curve (AUC) (Suhl et al 1986).

In renal transplant patients treated with immunosuppressive 6-thiopurine drugs, the main pharmacokinetic parameters of 6-mercaptopurine from azathioprine are volume of distribution (Vd), elimination rate constant (K_{el}), elimination half-life ($t\frac{1}{2}_{el}$), area under the plasma concentration–time curve (AUC), maximum concentration (C_{max}), and time of the maximum concentration (t_{max}). The inter- and intra-individual variations of these are very wide (Ohlman et al 1993) and although the significance of these variations on drug efficiency and toxicity is largely understood, the relationships between the different TPMTase phenotypes and 6-mercaptopurine bioavailability are unknown.

The purpose of this work was to validate an HPLC method for measurement of the plasma concentration of 6-mercaptopurine (after a test dose of azathioprine) and to calculate the pharmacokinetic parameters of this drug in a population of transplant subjects with high (or intermediate) TPMTase activity phenotypes.

We would then be able to determine the values of these parameters usually encountered and to compare these standard values with the pharmacokinetic values obtained for patients with low TPMTase activity phenotype.

Materials and Methods

Reagents
6-Mercaptopurine, 6-thioguanine and dithiothreitol were obtained from Sigma. Sodium hydroxide (NaOH), isoamyl alcohol (Uvasol), sulphuric acid (H_2SO_4), potassium dihydrogen phosphate (KH_2PO_4) and orthophosphoric acid (H_3PO_4) were from Merck. Phenylmercuric acetate was purchased from Aldrich, and toluene and acetonitrile (Chromasol) from S.D.S.

Stock solutions of 6-mercaptopurine (100 mg L^{-1}) and 6-thioguanine (200 mg L^{-1}) were prepared in NaOH (0·4 N). These solutions were used to prepare aqueous standard solutions of 6-thioguanine (1 mg L^{-1}).

The extraction solvent was a solution of phenylmercuric acetate (0·03%) and isoamyl alcohol (1%) in toluene.

Subjects, inclusion procedures and clinical and biological values
Pharmacokinetic parameters were calculated for 103 patients (73 male, 30 female), 12–62 years, 29–92 kg (mean 64·19 ± 13·3). All had received a renal allograft transplant between two and 19 years before the experiment. Currently used immunosuppressive treatment with azathioprine was stopped two weeks before the test, other immunosuppressive therapies being continued. No leucopenia or episodes of acute or chronic rejection were observed in the subjects during the three months before the test.

The subjects were known to have high or intermediate thiopurine methyl transferase activity (measured by a radiochemical method; Weinshilboum et al 1978) or by high-performance liquid chromatography (HPLC; Jacqz-Aigrain et al 1994). The main associated immunosuppressive treatment was with corticosteroids (10 mg day^{-1}) and cyclosporin (total blood concentrations 60–150 ng mL^{-1}).

The procedures were approved by the ethics committee of the C.H.U. of Dijon and explained to all the patients, who gave their informed consent to taking part in the study.

Azathioprine test doses and blood sampling
The first blood sample was taken, after fasting, at 0800 h; this was followed immediately by oral administration of the azathioprine test dose (50–175 mg, mean 123 mg, = 1·92 mg kg^{-1}) corresponding to 67·5 mg of 6-mercaptopurine (1·04 mg kg^{-1}). Serial plasma samples were collected 0·5, 1, 1·5, 2, 2·5, 3, 4, 5 and 6 h after drug ingestion. Plasma drug concentrations were measured by HPLC on the same day.

Chemical methods
Various methods were used for 6-mercaptopurine measurements in plasma: fluorimetry (Finckle 1967), gas chromatography (Bailey et al 1975) and HPLC. The HPLC method presented here is a modification of a method published elsewhere (Whalen et al 1985). We tried to simplify it and to make it more sensitive. After organic extraction in an alkaline layer with phenylmercuric acetate, chromatographic analysis of 6-mercaptopurine was

performed by reversed-phase HPLC. 6-Thioguanine (2-amino-6-mercaptopurine) was used as internal standard and detection was performed at 323 nm.

HPLC method

The mobile phase was KH_2PO_4 buffer (10 mM, pH 2·5) containing 4% acetonitrile and 150 mg L^{-1} dithiothreitol. HPLC was performed with a Waters M600 (Millennium) pump; the flow rate was 1·0 mL min^{-1} at a pressure of 2000 psig (Waters automatic injector). A Hibar 30 mm × 4 mm i.d. reversed-phase column (Merck), a Bondapak C_{18} column with 35–50-μm particles (Waters) and a LiChrospher 250 mm × 4 mm i.d. 100 RP-18 column with 5-μm particles (Merck) were connected in a series and used at room temperature. A spectrophotometric photodiode array detector (Waters 996) was operated at a wavelength of 323 nm.

Whole blood (5 mL) was collected in heparinized tubes. The tubes were centrifuged at 3000 rev min^{-1} (40 g) for 10 min. The assay requires 400 μL plasma. Analyses were performed immediately.

6-Thioguanine (5 mg mL^{-1}, 10 μL) and NaOH (0·5 N; 40 μL) were added to the sample, the extraction solvent (1·6 mL) was added and the mixture was vortex-mixed for 3 min and cen-

trifuged at 3000 g for 5 min. The clear organic layer was transferred to a second tube containing sulphuric acid (0·1 N; 250 μL). The tube was then vortex-mixed for 3 min, centrifuged at 3000 rev min^{-1} for 5 min, and the acid layer (100 μL) was injected into the chromatograph.

A typical chromatogram obtained from the plasma of a transplant patient is presented in Figure 1.

Calibration procedure

Five standard plasma samples were prepared by addition of aqueous standard solutions of 6-mercaptopurine (10, 25, 50, 75 and 100 ng mL^{-1}) and the standards were analysed and the 6-mercaptopurine to 6-thioguanine peak-area ratio was calculated. The working calibration curve was then plotted and used to determine the concentration of 6-mercaptopurine in the unknown samples (Figure 2).

Validation criteria

Coefficients of variation (CV, %) for repeatability were calculated from three pools of 20 standards containing 30 ng mL^{-1} 6-mercaptopurine in plasma. CV percentages for reproducibility were calculated from five pools of 10 plasma standards containing 0, 10, 30, 50 or 100 ng mL^{-1} 6-mercaptopurine over a period of 5 days.

Pharmacokinetic and statistical calculations

Various parameters (observed AUC and Cmax, tmax, $t\frac{1}{2}_{el}$, tlag (lag-time)) were calculated with Winonlin computer software using the classical one-compartment model with lag-time (Gauss–Newton algorithm):

$$C(t) = [D.f.K_{el}.(e^{-K_{el}} - e^{-K_{at}})]/V(K_a - K_{el}) \quad (1)$$

where $C(t)$ is the plasma concentration (mg L^{-1}) at time t; D is the dose (0·551 × azathioprine dose, mg); f is the fraction of the dose absorbed; K_{el} is the elimination rate constant (h^{-1}); and K_a is the absorption rate constant (h^{-1}). This model is valuable only if azathioprine is linearly transformed into 6-mercaptopurine (the molecular
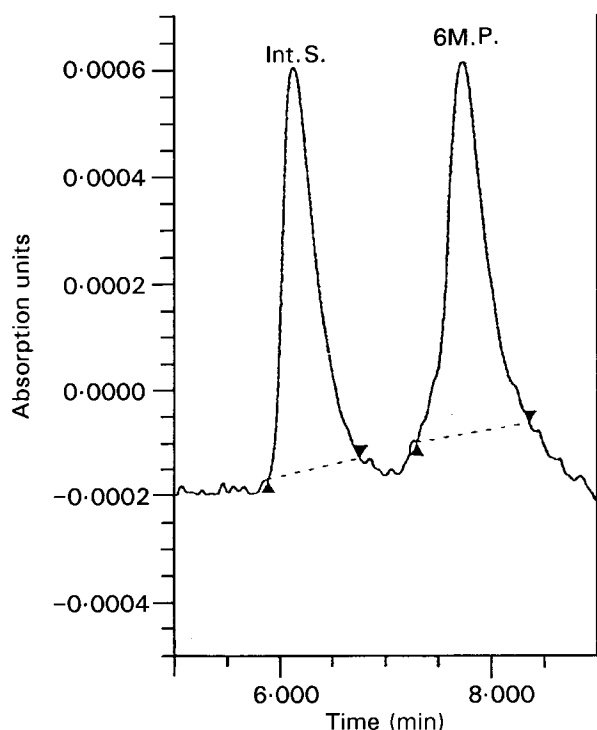

Figure 1. Typical chromatogram of 6-mercaptopurine (6M.P., retention time 6·227 min) in plasma (internal standard (Int.S.) 6-thioguanine, retention time 7·827 min).
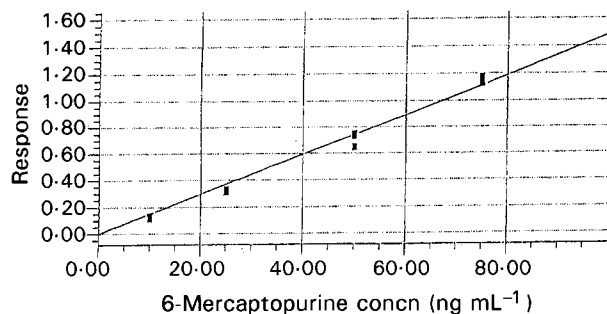

Figure 2. Calibration curve for HPLC determination of 6-mercaptopurine in plasma (using 6-thioguanine as internal standard). R = 0·9994; s.e.m. = 0·056.

conversion factor is 6-mercaptopurine = 0.551 × azathioprine).

Pharmacokinetic parameters

Stratification of the population. Stratified subgroups of patients were grouped according to the dose used: group A (n = 16) mean dose of azathioprine 0.96 mg kg⁻¹ (equivalent to 0.536 mg kg⁻¹ 6-mercaptopurine); group B (n = 34) mean dose of azathioprine 1.56 mg kg⁻¹ (equivalent to 0.86 mg kg⁻¹ 6-mercaptopurine); group C (n = 43) mean dose of azathioprine 2.2 mg kg⁻¹ (equivalent to 1.22 mg kg⁻¹ 6-mercaptopurine); and group D (n = 10) mean dose of azathioprine 2.93 mg kg⁻¹ (equivalent to 1.6 mg kg⁻¹ 6-mercaptopurine). We also grouped those subjects with the intermediate methylator phenotype (n = 6, TPMTase activity < 21 pmol h⁻¹ mg Hb⁻¹).

Total population of transplant patients. The mean dose of azathioprine (1.92 mg kg⁻¹) corresponds to 1.04 mg kg⁻¹ 6-mercaptopurine. The pharmacokinetic parameters were calculated using the mean of all the individual pharmacokinetic values calculated for the whole population (103 patients).

Statistical calculations

Data are presented as means ± s.e.m. (standard error of the mean); the estimated standard errors are based on linearization of the models. The Mann–Whitney U-test was used to compare values between groups.

Simulation

The Winonlin software was also used to compare the measured values of 6-mercaptopurine (the average plasma concentrations of 6-mercaptopurine measured at each time-point) with predicted values (theoretical time–concentration curve) calculated with the one-compartment model (Figure 3).
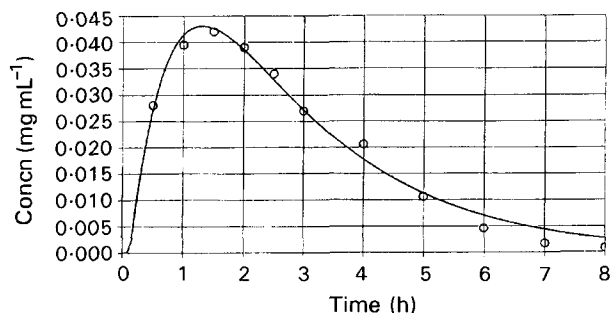

Figure 3. Plot of plasma 6-mercaptopurine concentration–time curve for 103 high or intermediate TPMTase activity phenotypes (○) compared with the predicted (simulated) curve (—).

Results

Validation criteria

Coefficients of variation (CV, %) for repeatability were 8.7, 8.4 and 9.4%. CV percentages for reproducibility were 0, 7.7, 8.2, 6.3 and 2%. The limit of detection was between 1 and 2.5 ng mL⁻¹ (CV 10%). The limit of quantification was measured up to 100 ng mL⁻¹ (CV 10%). The extraction procedure resulted in very good selectivity—other metabolites of 6-mercaptopurine were not detected and the addition of a variety of drugs (penicillin, gentamycin, cyclosporin, corticosteroids) did not affect the analysis of 6-mercaptopurine.

The mean values of pharmacokinetic parameters in stratified subgroups

The mean values of calculated pharmacokinetic parameters are presented in Table 1; there were no significant differences among the dose-stratified subgroups and the values measured for the population of intermediate methylators (I) were identical.

Table 1. Mean values of 6-mercaptopurine pharmacokinetic parameters after azathioprine test dose in dose-stratified groups (A, B, C, D) and in intermediate methylators (I).

	Mean azathioprine dose				
	A (n = 16) 0.96 mg kg⁻¹	B (n = 34) 1.56 mg kg⁻¹	C (n = 43) 2.2 mg kg⁻¹	D (n = 10) 2.92 mg kg⁻¹	I (n = 6) 1.96 mg kg⁻¹
AUC (ng mL⁻¹h)	126 ± 70	186 ± 130	200 ± 150	233 ± 109	154 ± 136
K_{el}	2.14 ± 2.10	2.02 ± 0.80	1.79 ± 0.73	1.86 ± 0.90	1.54 ± 4.60
$t_{\frac{1}{2}el}$ (h)	0.56 ± 0.41	0.41 ± 0.20	0.46 ± 0.21	0.46 ± 0.21	0.45 ± 0.15
t_{lag} (h)	0.50 ± 0.33	0.56 ± 0.43	0.42 ± 0.32	0.41 ± 0.57	0.49 ± 0.40
t_{max} (h)	1.37 ± 0.60	1.56 ± 0.80	1.45 ± 0.54	1.47 ± 0.68	1.88 ± 1.40
C_{max} (ng mL⁻¹)	47 ± 27	57 ± 46	56 ± 30	63 ± 36	28 ± 19

AUC, area under the plasma concentration–time curve; K_{el}, elimination rate constant; $t_{\frac{1}{2}el}$, elimination half-life; t_{lag}, lag time; C_{max}, maximum plasma concentration; t_{max}, time to maximum plasma concentration.

Population studies—distribution of calculated parameters

Figure 4 shows $t^{\frac{1}{2}}_{el}$ probit curves for 6-mercapto-purine in the population of azathioprine-treated patients. Figure 5 shows the same distributions using the normal test variable plot as suggested by Endrenyi & Patel (1991). In these representations, even though the distribution (for $t^{\frac{1}{2}}_{el}$) seems to be bimodal, the plotted values simply reflect the non-uniformity of the study population.

The mean values of pharmacokinetic parameters measured for the 103 subjects are listed in Table 2.

The correlation (R = 0·983) found between experimental (observed) and calculated (predicted) values indicated the validity of pharmacokinetic model used.

Discussion

The results of our study of transplant patients with high or intermediate TPMTase activity phenotypes can be summarized as follows. After oral administration of the test dose of azathioprine no significant difference was found between the
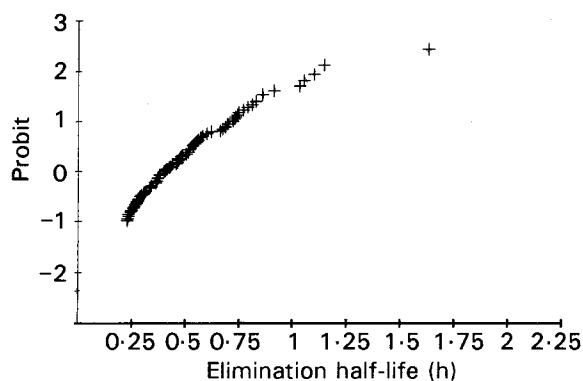

Figure 4.   Probit representation of the half-life of 6-mercaptopurine in the population of transplanted patients (103 subjects).
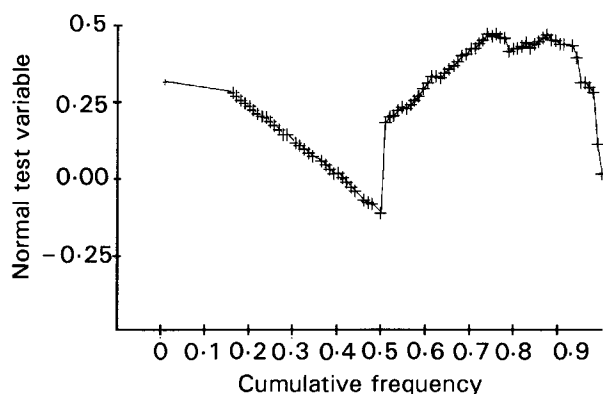

Figure 5.   Normal test variable plot representation of the elimination half-life of 6-mercaptopurine in the population of transplanted patients (103 subjects).

Table 2.   6-Mercaptopurine pharmacokinetic parameters in the study population (103 kidney transplanted patients) after azathioprine test dose.

AUC (ng mL^{-1} h)	190 ± 140
K_{el}	1·92 ± 1·00
$t^{\frac{1}{2}}_{el}$ (h)	0·46 ± 0·25
t_{lag} (h)	0·47 ± 0·40
t_{max} (h)	1·48 ± 0·64
C_{max} (ng mL^{-1})	60 ± 40

UC, area under the plasma concentration–time curve; K_{el}, elimination rate constant; $t^{\frac{1}{2}}_{el}$, elimination half-life; t_{lag}, lag time; C_{max}, maximum plasma concentration; tmax, time to maximum plasma concentration.

pharmacokinetic parameters measured in the dose-stratified subgroups or for the population of intermediate methylator phenotypes. The values of K_{el} and $t^{\frac{1}{2}}_{el}$ were normally distributed in the population and pharmacokinetic parameters were not dose-dependent.

No real anti-mode could be demonstrated in this distribution.

Because the calculated mean values are representative of azathioprine metabolism in allograft transplant patients with high or intermediate TPMTase activity phenotype, they can be seen as standard values for these parameters in this population.

These results for 6-mercaptopurine elimination parameters corroborate other results for metabolism both of azathioprine (Maddoks 1978; Ding et al 1980; Lin et al 1980; Odlind et al 1986; Chan et al 1990) and of 6-mercaptopurine (Suhl et al 1986). Wide inter- and intra-individual variations of the pharmacokinetic parameters were observed among transplant patients (Table 3). However, in these experiments the TPMTase activity of the patients was not phenotyped because it was unknown whether leucopenia episodes had occurred.

In another study Escousse et al (1995) measured the systemic exposure (AUC) and other pharmacokinetic parameters for 6-mercaptopurine in a patient with a low TPMTase activity phenotype (TPMTase = 0). In this patient the haematological toxicity of the drug was clearly augmented (as classically demonstrated), as was the systemic exposure to 6-mercaptopurine (AUC increased tenfold).

If these conventional 6-mercaptopurine pharmacokinetic data could be extrapolated, it would be possible to measure azathioprine hypersensitivity in individual patients, currently considered as therapeutic orphans as far as immunosuppressive treatment with azathioprine is concerned, and so to reduce the doses of azathioprine used, thus limiting haematological toxicity.

Table 3. Elimination half-life values of azathioprine, and of 6-mercaptopurine
derived from azathioprine, in kidney transplant recipients.

Azathioprine dose (mg)	Elimination half-life	Patient population	Reference
50–100 (intravenous)	62·6 ± 24·4	8	Ding et al (1980)
50 (intravenous)	114	30	Maddoks (1978)
100–150 (intravenous)	38·4 ± 16·7	10	Lin et al (1980)
180 (mean; oral)	114 ± 36	6	Chan et al (1990)
100	73·6 ± 58	15	Odlind et al (1986)

Moreover, pharmacokinetic parameters of 6-mercaptopurine (particularly AUC) could also be indicators of the therapeutic immunosuppressive activity of azathioprine as shown by the cytotoxic efficiency of the drug. Unfortunately, in-vitro measurements of immunosuppressive action (the rosette-inhibiting effect) have been unable to prove this type of correlation (Odlind et al 1986).

Azathioprine has previously been shown to be a successful immunosuppressive agent, but there is a need for pharmacokinetic markers of activity and toxicity, because, as noted previously (Ohlman et al 1993) in many transplant patients the drug is not used optimally, for fear of its myelotoxic effect. After administration of a test dose of azathioprine, measurement of TPMTase activity and of systemic exposure to 6-mercaptopurine (AUC) might be good markers of the toxicity of this drug and useful for prediction for dose reduction.

References

Bailey, D. G., Wilson, T. W., Johnson, G. E. (1975) A gas chromatographic method for measuring 6-mercaptopurine in serum. J. Chromatogr. 11: 305–311

Brooks, R. S., Dorr, R. T., Durie, B. G. M. (1982) Interactions of allopurinol with 6-mercaptopurine and azathioprine. Biomedicine 36: 217–222

Chan, G. L. C., Canafax, D. M., Johnson, C. A. (1987) The therapeutic use of azathioprine in renal transplantation. Pharmacotherapy 7: 165–167

Chan, G. L. C., Erdman, G. R., Gruber, S. A., Matas, A. J., Canafax, D. M. (1990) Azathioprine metabolism: pharmacokinetics of 6-mercaptopurine, 6-thiouric acid and 6-thioguanine nucleotides in renal transplant patients. J. Clin. Pharmacol. 30: 358–363

Ding, T. L., Gamberatoglio, J. G., Amend, W. J. C., Birnbaum, J., (1980) Azathioprine (AZA) bioavailability and pharmacokinetics in kidney transplant patients. Clin. Pharmacol. Ther. 27: 250

Elion, G. B. (1967) Biochemistry and pharmacology of purine analogues. Fed. Proc. 26: 898–903

Endrenyi, L. E., Patel, M. (1991) A new sensitive graphical method for detecting deviations from the normal distribution of drug response: the NTV plot. Br. J. Clin. Pharmacol. 32: 159–166

Escousse, A., Rifle, G., Sgro, C., Mousson, C., Zanetta, G., Chevet, D. (1995): Azathioprine toxicity, 6-mercaptopurine bioavailability, and the 'poor' 6-thiopurine methylator phenotype. Eur. J. Clin. Pharmacol. 48: 309–310

Finkle, J. M. (1967) A fluorimetric method for the estimation of 6-mercaptopurine in serum. Ann. Biochem. 21: 362–371

Jacqz-Aigrain, E., Bessa, E., Medard, Y., Mircheva, Y., Vilmer, E. (1994) Thiopurine methyltransferase activity in a French population: HPLC assay conditions and effects of drugs on inhibition. Br. J. Clin. Pharmacol. 38: 1–8

Lennart, L., Van Loon, J. A., Weinshilboum, R. M. (1989) Pharmacogenetics of acute azathioprine toxicity: relationship to thiopurine methyltransferase genetic polymorphism. Clin. Pharmacol. Ther. 46: 149–154

Lennart, L., Lilleyman, J. S., Van Loon, J. A., Weinshilboum, R. M. (1990) Genetic variations in response to 6-mercaptopurine for childhood acute lymphoblastic leukemia. Lancet 336: 225–229

Lin, S. N., Jessup, K., Floyd, M., Wang, T. F., Van Boren, C. T. (1980) Quantification of plasma azathioprine and 6-mercaptopurine levels in renal transplant patients. Transplantation 29: 290–294

Maddoks, J. L. (1978) Clinical pharmacological observation on azathioprine in kidney transplant patients. Clin. Sci. Mol. Med. 55: 20P

Odlind, B., Hartvig, P., Lindstrom, B., Lonnerholm, G., Tufueson, G. (1986) Serum azathioprine and 6-mercaptopurine levels and immunosuppressive activity after azathioprine in uremic patients. Int. J. Immunopharmacol. 8: 1–11

Ohlman, S., Lafolie, P., Lindholm, A., Liliemark, J., Tyden, G., Peterson, C. (1993) Large inter-individual variability in bioavailability of azathioprine in renal transplant recipients. Clin. Transplant. 7: 65–70

Sauer, H., Hantke, U., Wilmans, W. (1988) Azathioprine lymphotoxicity: potential lethal damage by its imidazole derivatives. Drug Res. 38: 820–824

Schwartz, R. S., Eisner, A., Dameshek, W. (1959) The effect of 6-mercaptopurine on primary and secondary immune responses. J. Clin. Invest. 38: 1394–1403

Suhl, H., Koren, G., Whalen, C., Soldin, S., Ziporski, A., Greenberg, M. (1986) Pharmacokinetic determinants of 6-mercaptopurine myelotoxicity and therapeutic failure in children with acute lymphoblastic leukemia. Clin. Pharmacol. Ther. 40: 604–609

Van Scoik, K. G., Johnson, C. A., Porter, W. R. (1985) The pharmacology and metabolism of the thiopurine drugs: 6-mercaptopurine and azathioprine. Drug Metab. Rev. 16: 157–174

Weinshilboum, R. M., Raymond, F. A., Pazmino, P. A. (1978) Human erythrocyte thiopurine methyltransferase: radiochemical microassay and biochemical properties. Clin. Chim. Acta 85: 323–333

Whalen, C. E., Tamary, H., Greenberg, M., Ziporski, A., Soldin, S. J. (1985) Analysis of 6-mercaptopurine in serum or plasma using high performance liquid chromatography. Ther. Drug Monit. 7: 315–320