Congenital thiopurine methyltransferase deficiency and 6-mercaptopurine toxicity during treatment for acute lymphoblastic leukaemia

L Lennard, B E S Gibson, T Nicole, J S Lilleyman

Abstract

Two children with acute lymphoblastic leukaemia (ALL) taking daily 6-mercaptopurine as part of a national UK therapeutic trial repeatedly developed profound myelosuppression on 25% of the standard protocol dose. Both were found to have undetectable intracellular activity of thiopurine methyltransferase (TPMT), an enzyme controlling one of the major alternative catabolic pathways of 6-mercaptopurine, and both produced higher concentrations of cytotoxic drug metabolites at 10–25% of the protocol dose than other patients taking 100%.

It is supposed that these patients represent the 0·33% of the normal population constitutionally lacking TPMT. It is important to recognise such individuals both to avoid fatal bone marrow failure through inadvertent overdosage, and to be reassured that an adequate drug effect can be achieved at around 10% of the standard dose.

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University of Sheffield Department of Medicine, Section of Pharmacology and Therapeutics, Royal Hallamshire Hospital, Sheffield S10 2JF
L Lennard

Department of Haematology, Royal Hospital for Sick Children, Glasgow
B E S Gibson

Department of Paediatrics, Yeovil District Hospital, Somerset
T Nicole

University of Sheffield Department of Paediatrics, Section of Paediatric Haematology, The Children's Hospital, Sheffield
J S Lilleyman

Correspondence to:
Dr Lennard.

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Inherited as a genetic trait, constitutional absence of thiopurine methyltransferase (TPMT) activity occurs in 0·33% of the white population[1] and so could be expected to occur in a similar proportion of children being treated with 6-mercaptopurine for acute lymphoblastic leukaemia (ALL).

The enzyme is responsible for S-methylation of 6-mercaptopurine and its initial nucleotide metabolites, and competes with two other 6-mercaptopurine metabolic pathways, modulated by hypoxanthine phosphoribosyl transferase (HPRT) and xanthine oxidase respectively.

6-Mercaptopurine is a prodrug with no intrinsic cytotoxic activity. It has to be processed within cells to produce cytotoxic metabolites, the most important being the 6-thioguanine nucleotides at the end of the HPRT pathway. Intracellular concentrations of these metabolites correlate with granulocytopenia during 6-mercaptopurine treatment,[2,3] and low concentrations seen during treatment of ALL appear to be associated with a higher risk of relapse.[4]

6-Mercaptopurine is broken down by xanthine oxidase, and it has long been known that the concurrent administration of allopurinol (a xanthine oxidase inhibitor) with 6-mercaptopurine potentiates cytotoxicity.[5,6] Less well recognised, but no less clinically important, low or absent TPMT activity is also associated with grossly raised 6-thioguanine nucleotide concentrations and profound myelosuppression after only a brief exposure to thiopurine drugs.[7] The problem has been seen in occasional adults on immunosuppressive treatment with azathioprine (a 6-mercaptopurine prodrug),[7,8] and has been described in children with ALL.[9,10]

We have recently encountered two children in the UK ALL trial series who gave an opportunity to assess intracellular drug metabolites in such circumstances and to explore how best the rare child with ALL and constitutional TPMT deficiency might be managed. The values were compared with a well studied unselected cohort of children with intermediate or high TPMT activity.

Methods and reference ranges

REFERENCE POPULATION

Ranges for 6-methylmercaptopurine metabolites (MeMPs), 6-thioguanine nucleotides, and TPMT activity were taken from a consecutive cohort of 25 children with ALL from two treatment centres. Assays were taken during continuation chemotherapy on current UK ALL protocols where prolonged treatment with daily 6-mercaptopurine is coupled with weekly methotrexate and monthly vincristine plus steroids. Samples were taken at routine venepuncture at a time when the patients had been prescribed 100% standard protocol dose of 6-mercaptopurine (75 mg/m^2) for at least four weeks or more and when no red cell transfusions had been given for at least three months.

ASSAYS

Red blood cell concentrations of MeMPs, products of the TPMT reaction, and 6-thioguanine nucleotides were measured as previously described.[11] Red blood cell TPMT activity was measured by a slight modification of the method of Weinshilboum *et al.*[11,12] One unit of TPMT activity represents the formation of 1 nmol 6-methylmercaptopurine per hour of incubation per ml of packed red blood cells.

REFERENCE VALUES

6-Thioguanine nucleotide concentrations ranged from 144-1263 pmol/8×10^8 red blood cells (median 335), MeMP concentrations ranged from 0·25–22·5 nmol/8×10^8 red blood

6-Mercaptopurine dosages, 6-thioguanine nucleotide concentrations (pmol/8×10⁸ red blood cells) MeMP concentrations (nmol/8×10⁸ red blood cells), and TPMT activities (units/ml red blood cells) in the reference population (control group) compared with patients 1 and 2 (100% 6-mercaptopurine=75 mg/m²).

% 6-Mercaptopurine	Range (median) 6-thioguanine nucleotides	Range (median) MeMPs	Range (median) TPMT
Control group			
100%×4 weeks	144–1263 (335)	0·25–22·5 (4·36)	9·2–25·1 (17·5)
Patient 1			
25%×2 weeks	1832	Nil	
10%×3 weeks	960	Nil	
10%×6 weeks	1701	Nil	<0·74
Patient 2			
50%×1 week	1601	Nil	
0%×1 week	2650	Nil	
25%×2 weeks	2250	Nil	
0%×3 weeks	628	Nil	<0·074

cells (median 4·36), and TPMT values ranged from 9·2–25·1 units (median 17·5).

Case reports

PATIENT 1

A 3 year old boy developed 'common' ALL and was treated according to the then current UK protocol, UKALL XA. He entered remission uneventfully, and started 6-mercaptopurine treatment on schedule at 100% dose (75 mg/m²). After 19 days he developed neutropenia (neutrophil count $0·2\times10^9$/l) and thrombocytopenia (platelet count 12×10^9/l) became unwell and needed admission to hospital. His counts recovered during the ensuing six weeks, and 6-mercaptopurine was restarted at 50% dose rising to 75% over four weeks. At this stage his counts plummeted again (neutrophils $0·5\times10^9$/l, platelets 12×10^9/l). Subsequently he never tolerated more than 25% of the protocol dose of 6-mercaptopurine.

6-Mercaptopurine metabolites were measured on three occasions: after two weeks at 25% protocol dose (6-thioguanine nucleotides 1832 pmol/8×10⁸ red blood cells, MeMPs nil), after three weeks at 10% protocol dose (6-thioguanine nucleotides 960 pmol/8×10⁸ red blood cells, MeMPs nil), and after six weeks at 10% protocol dose (6-thioguanine nucleotides 1701 pmol/8×10⁸ red blood cells, MeMPs nil). TPMT activity was recorded at <0·74 units, the lowest detectable activity in the assay used (table).

He settled to a tolerated 10% of protocol dose, though his platelet count was $<100\times10^9$/l for two months on one occasion, and treatment had to be interrupted four more times when his platelets fell to $<50\times10^9$/l and/or his neutrophils below $0·5\times10^9$/l. He completed his treatment and continues in his first remission three years from diagnosis.

PATIENT 2

A 12 year old girl developed 'common' ALL with an initial white cell count of 196×10^9/l and was treated according to the protocol of UKALL XI. At the start of 6-mercaptopurine treatment she was prescribed full protocol dose and four weeks later was profoundly neutropenic (neutrophil count zero) and thrombocytopenic (platelet count 129×10^9/l). Her counts recovered in a month and she was reintroduced to 6-mercaptopurine at a starting dose of 25% rising to 50% over three weeks. Pancytopenia recurred. Subsequently she never tolerated 6-mercaptopurine for more than three weeks and rarely received more than 25% of the standard dose.

6-Mercaptopurine metabolites were measured on four occasions: after 1 week at 50% of the standard dose (6-thioguanine nucleotides 1601 pmol/8×10⁸ red blood cells, MeMPs nil), after one week at 25% (6-thioguanine nucleotides 2250 pmol/8×10⁸ red blood cells, MeMPs nil), and also one and three weeks after withdrawing treatment at 25% (6-thioguanine nucleotides 2650 and 628 pmol/8×10⁸ red blood cells, MeMPs nil and nil, respectively). TPMT was assayed at <0·74 units, below the limit of assay (table).

The patient settled to a cycle of daily 6-mercaptopurine for three weeks at 25% of the standard dose usually followed by a cytopenia induced 3–4 week gap. She continues in her first remission 1·5 years from diagnosis.

Discussion

The two children described illustrate the problems of thiopurine drug treatment in patients constitutionally lacking TPMT. Both developed severe myelotoxicity on standard doses of 6-mercaptopurine, and both were subsequently able to tolerate only very small doses of the drug. Despite this, both consistently showed high concentrations of intracellular 6-thioguanine nucleotides, higher than those seen in normal children taking full doses.

The assumption that they lacked TPMT was based on direct assay of the enzyme and the repeated failure to detect any MeMPs – the product of the enzyme. Although MeMPs fluctuate widely in normals on 6-mercaptopurine, they are never undetectable.

TPMT deficiency occurs in approximately 0·33% of the UK population, and some 400 children each year develop ALL. Current treatment schedules run for two years, so at any one time around 800 patients will be on continuous 6-mercaptopurine. Statistically two or three will be TPMT deficient, so the two patients in this report probably represent a nationwide experience. Because of their rarity, it is possible that paediatric oncologists may not encounter a TPMT deficient child for many years, but it is important that they are aware of the possibility to avoid potentially life threatening marrow toxicity when giving 6-mercaptopurine. The same would apply to children receiving azathioprine for any reason.

The concentrations of 6-thioguanine nucleotides seen in the two patients are reassuringly high, despite the fact that they were taking what might be regarded as homoeopathic doses of 6-mercaptopurine. It is likely that these cytotoxic metabolites, as chief mediators of the drug's antineoplastic effect, can be maintained at satisfactory concentrations on tiny doses in such children. They accumulate slowly in cells over several days and then disappear at the same rate, as shown

by patient 2, who was studied at one and two weeks after interrupting treatment. TPMT deficient children are never likely to reach 'steady state' for 6-thioguanine nucleotides, but their concentration can probably be kept within reasonable limits by judicious titration against the neutrophil and platelet count.

The fact that TPMT deficient children do not produce intracellular MeMPs may be unimportant as far as the cytotoxic effect of 6-mercaptopurine is concerned, though the precise role and kinetics of those metabolites is still not clear. There appears to be an inverse correlation between 6-thioguanine nucleotides and MeMPs,[13] but they may interact in cytotoxicity, as children without MeMPs appear to tolerate much higher concentrations of 6-thioguanine nucleotides. This is apparent when 6-thioguanine, a direct source of the nucleotides, is offered as an alternative to 6-mercaptopurine.[14]

The most important point about TPMT deficiency is to avoid toxicity with 6-mercaptopurine and azathioprine. But it is also important to appreciate that an apparently adequate drug effect can be achieved on markedly reduced doses.

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