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High-performance liquid chromatographic assay of metabolites of thioguanine and mercaptopurine in capillary blood

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Abstract

The main metabolites of the cytotoxic drugs thioguanine (6TG) and mercaptopurine (6MP) can be measured conveniently in red blood cells (RBC). Isolation of RBC, however, is laborious and requires some milliliters of blood. This HPLC assay allows measurements of thiopurine metabolites in very small blood samples obtained from the finger-tip. The metabolites, derivatives of 6TG and methylmercaptopurine (6MeMP), were extracted and hydrolized with perchloric acid to liberate the corresponding base. 6MeMP is completely transformed under these conditions to 4-amino-5-(methylthio)carbonyl imidazole. The chromatographic separation of 6TG and this imidazole was performed in a single run under isocratic conditions within 10 min using a 70 mm column. The quantification limit was 0.5 nmol/ml for 6TG and 3 nmol/ml blood for 6MeMP. The accuracy was 83% for 6TG (CV = 3%) over the concentration range of 0.5–20 nmol/ml blood and 102% (CV = 4%) for 6MeMP over the range of 3–150 nmol/ml blood. The intra-assay CV ranged from 5.4 to 7.4% for 6TG and from 6.2 to 10.6% for 6MeMP. The inter-assay CV was 7.5 and 9.5% in a pooled blood sample. The levels in RBC in whole blood were nearly coincident with those obtained in separated RBC, isolation of RBC therefore is not necessary for these measurements, if the drugs are given per os in the day before blood sampling. The concentration of 6MeMP nucleotides is more dependent on the given 6MP dose than the concentration of 6TG nucleotides. Intraindividual variations were small at unchanged drug doses, interindividual metabolite concentrations were highly variable.

Keywords: Thioguanine; Mercaptopurine

1. Introduction

The antimetabolites thioguanine (6TG) and mercaptopurine (6MP) have been used in the treatment of acute leukemia for more than four decades; 6MP became the drug of choice for maintenance therapy of acute lymphoblastic leukemia, whereas 6TG is used primarily in acute myelogenous leukemia [1]. Our cooperative study for treatment of acute lymphoblastic leukemia in childhood, COALL-92, was

Abbreviations: 6TG, thioguanine; 6MP, mercaptopurine; 6MeTG, 6-methyl thioguanine; 6MeMP, 6-methylmercaptopurine; TGMP, thioguanosine monophosphate; TIMP, thioinosine monophosphate; MeTIMP, 6-methyl thioinosine monophosphate; DTT, dithiothreitol

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the first to randomize patients to receive either 6MP or 6TG in maintenance treatment to investigate whether 6TG offers a therapeutic advantage over 6MP [2–5]. Similar trials meanwhile were started in the US by the Childrens's Cancer Group and in the UK by the Medical Research Council's Childhood Leukaemia Working Party.

Both thiopurines are prodrugs and have to be converted into active metabolites in order to exert cytotoxicity. As shown in detail by Aarbakke et al. [4], in a first step the corresponding ribonucleotides thioguanosine monophosphate (TGMP) and thioinosine monophosphate (TIMP) are formed. Via two additional enzymatic steps, TIMP is also converted to TGMP which can be finally incorporated into DNA and RNA in its (deoxy) triphosphate form. Furthermore, TIMP as well as other metabolites and the parent drugs 6MP and 6TG are also substrates for the enzyme thiopurine methyltransferase (TPMT) which catalyzes the methylation of the sulfur at position 6.

It is commonly assumed that the incorporation of the thioguanine nucleotides (TGN) into DNA is of decisive importance for the cytotoxic effect of 6MP as well as of 6TG [6,7]. The role of the methylated nucleotides is discussed controversially. However, from our study, it seems likely that the cytotoxicity at least of 6MP is only partly mediated through TGN [5]. In vitro studies have shown that methylation contributes to the cytotoxic properties of mercaptopurine, whereas thioguanine is inactivated primarily by TPMT [8,9]. Inhibition of purine de novo synthesis by MeTIMP as well as the inhibition of inosine monophosphate dehydrogenase by TIMP [1] could be additional mechanisms of action.

Crucial in every case is the concentration of active metabolites in the target cells. Since leukemic cells are not available for measurements once remission has been achieved the determination of the formed nucleotides in red blood cells (RBC) (which do not synthesize nucleic acids and therefore accumulate the metabolites) has to serve as a substitute. Metabolite concentrations in RBC allows certain conclusions as to treatment intensity [10–12], clinical outcome [13,14], and individual compliance [15,16].

Several HPLC methods have been described for the quantitative determination of thiopurine metabolites in RBC. In general, the bases hydrolytically liberated from the nucleotides are measured and chromatographic systems have been optimized for the separation of 6TG/methylmercaptopurine (6MeMP) [5,17,18] as well as for 6MeTG [5,19,20]. In all these methods RBC are separated before measurement of intracellular metabolite concentrations, requiring some milliliters of blood in every case. Regular collections of larger blood samples, however, may be difficult especially in younger children. Furthermore, separation and washing of RBC is very laborious and time consuming. In this study, we have investigated whether this isolation step is necessary indeed and we present now a rapid and simple procedure for the quantitative determination of thiopurine metabolites, specifically 6TG and 6MeMP based derivatives, in capillary blood collected from the finger-tip.

2. Experimental

2.1. Chemicals

The thiopurines and dithiothreitol (DTT) were obtained from Sigma, Hank's balanced salt solution (HBSS) from Life Technologies (Paisley, UK) and all other chemicals in analytical grade from Merck (Darmstadt, GFR). Purified water was produced with Purelab Plus (USF Seral, Ransbach, GFR).

2.2. Preparation of samples

Capillary blood from the finger-tip is collected in EDTA tubes (Kabe, Nürnbrecht, GFR) for routine analyses; for this investigation larger blood samples were additionally obtained with informed consent by venous puncture for the separation of RBC and plasma as described earlier [5]. RBC were counted (Cell-Dyn 1600; Abbott, Wiesbaden, GFR) and 25 μl aliquots of washed RBC, blood, or plasma were stored refrigerated at −20 °C. Refrigeration of RBC and blood is necessary in every case for the lysis of the cells before analysis.

Three hundred and fifty-five microliters of 6 mM DTT in HBSS were added to the thawed samples which were heated thereafter for 10 min at 37 °C and then deproteinized with 20 μl of perchloric acid (60%, final concentration 0.5 M). After 10 min the supernatant was centrifuged, transferred to an Eppendorf Safe lock tube and heated for 2 h at 100 °C

in order to achieve hydrolysis of nucleosides and nucleotides. 6MeMP is degraded under these conditions to 4-amino-5-(methylthio)carbonyl imidazole [21].

2.3. Preparation of standards

DTT was dissolved in purified water (0.2 M), the thiopurines were dissolved in 0.1N NaOH (1 mg/ml) and further diluted with water (1:100). All stock solutions were stored at −20 °C. The final concentrations of the aqueous HPLC calibration standards were 1.2 μM (6TG), 6.0 μM (6MeMP, as the imidazole), and 5 mM (DTT) in 0.5 M $HClO_4$. To achieve the formation of 4-amino-5-(methylthio)carbonyl imidazole, the calibration standard of 6MeMP was heated for 2 h at 100 °C in 0.5 M $HClO_4$ like the sample extracts.

2.4. Assay validation

The accuracy was determined by adding known amounts of 6TG (final concentration 0.5–20 nmol/ml) and 6MeMP (3–150 nmol/ml) to whole blood (pooled from patients not receiving thiopurines) which was then refrigerated at −20 °C overnight and analyzed as described. At every concentration five measurements were performed to determine intra-assay variation. A pooled blood sample from patients under thiopurine treatment was divided up to 25 μl aliquots, refrigerated and subsequently used for inter-assay experiments at 10 different days.

2.5. Chromatography

The HPLC system (Thermoquest, Egelsbach, GFR) consisted of the pump (P 200), the autosampler (AS 300) with Microvials (100 μl) and the variable UV-Vis detector (UV 2000), set at 342 nm for 6TG and switched at 300 nm for the detection of the imidazole. All separations were performed at room temperature on a reversed phase column (Nucleosil 120, C18, particle size 3 μm, 70 mm × 3 mm with gard cartridge; CHROMCARD®, Macherey & Nagel, Düren, GFR). The flow rate was 0.5 ml/min, the injection volume 25 μl (or 50). The mobile phase consisted of sodium dihydrogen phosphate (0.01 M), adjusted to pH 2.7 with phosphoric acid before addition of 2.4% (v/v) acetonitrile. In earlier experiments a larger column size (125 mm × 4.6 mm, 20 mm guard cartridge; GAT Analysentechnik, Bremen, GFR) was used [5].

2.6. Patients

Study COALL-92 was a multicenter treatment study for children with acute lymphoblastic leukemia aged from 1 to 18 years. Prior to maintenance therapy the patients were randomized to receive either daily 6MP or 6TG p.o.; methotrexate (MTX) p.o. was given weekly in both arms. The drug doses were adjusted in order to maintain a WBC count between 2 and 3 nl^{-1}. The starting dose for 6MP and 6TG was 50 and 20 mg/m^2 for MTX giving a desired ratio of 2.5 between 6MP/6TG and MTX [5]. The thiopurines were taken in the evening and blood sampling was done (bi) weekly next day. All samples were collected in our hospital.

3. Results

3.1. Hydrolysis

As (methyl-)thioguanine and methylmercaptopurine nucleotides are not commercially available, TIMP was used to determine the optimal time for hydrolysis. Within 60 min the corresponding base 6MP was liberated completely from this nucleotide (data not shown). This time is also sufficient to hydrolyse TGN in patient samples, the measured 6TG concentration was not increasing thereafter. The complete transformation of 6MeMP to the imidazole, however, requires 120 min under the hydrolytic conditions described. After this time no more 6MeMP is detectable in the calibration standard and the maximum peak height of the imidazole has been reached in the standard and in patient samples. This time can be shortened to 90 min by the addition of the two-fold volume of perchloric acid. An additional unidentified compound interfering with the imidazole was observed with DTT concentrations lower than 5 mM.

3.2. Chromatography

Fig. 1 shows the chromatogram of the standard mix, of a substrate blank, and of a pooled hydrolysed blood extract from patients receiving 6MP.

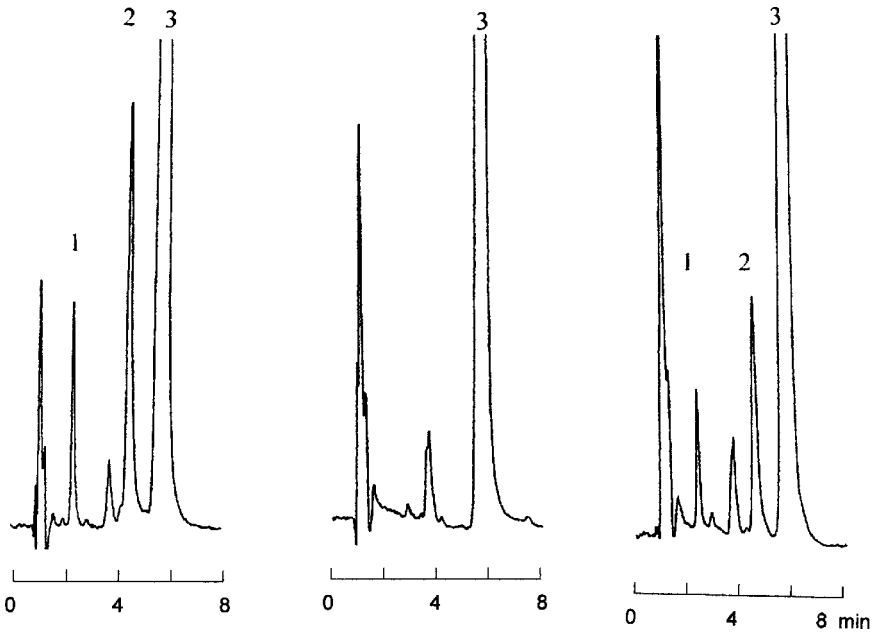

Fig. 1. Chromatographic separation of thioguanine and methylmercaptopurine in hydrolysed blood extracts. Chromatographic conditions: Nucleosil 120–3, C18, 70 mm × 3 mm with guard cartridge. The mobile phase consisted of phosphate buffer pH 2.7 with 2.4% acetonitrile. Flow 0.5 ml/min, injection volume 25 μl. Detection at 342 nm, switched to 300 nm at 3.4 min. (a) Standard mixture; (b) blank hydrolysed blood extract; (c) hydrolysed blood extract (pooled sample from patients under 6MP therapy). 1: thioguanine (2.4 min); 2: 4-amino-5-(methylthio)carbonyl imidazole (hydrolysis product of 6-methylmercaptopurine; 4.6 min); 3: dithiothreitol.

6TG and 4-amino-5-(methylthio)carbonyl imidazole can be separated in a 70 mm column under isocratic conditions (phosphate buffer pH 2.7 with 2.4% acetonitrile) within 10 min. The detector was set at 342 nm for measurement of 6TG (RT = 2.4 min) and switched to 300 nm before elution of the imidazole (4.5 min).

The limit of detection (signal to noise ratio of 3) was 1 pmol for 6TG and 2 pmol for 6MeMP, the limits of quantitation (CV < 15%) were 0.5 and 3 nmol/ml blood with an injection volume of 25 μl. The RBC counts in the measured samples were 2 to 5.0×10^9 ml^{-1} (median 3.8). Related to the highest RBC count the limit of quantitation would be 100 pmol/10^9 RBC for 6TG and 600 pmol/10^9 RBC for 6MeMP with the given injection volume.

3.3. Assay validation

Mean recoveries of 83% for 6TG (CV = 3%) and 102% for 6MeMP (CV = 4%) were found under these conditions over the concentration range, the intra-assay variation ranged from 5.4 to 7.4% (6TG) and 6.2 to 10.6% (6MeMP) (Table 1). The inter-assay variation in the pooled patient sample was 7.5% (6TG) and 9.1% (6MeMP).

3.4. Metabolite concentrations in plasma versus whole blood

In 10 samples from each 6TG- and 6MP-treated children the metabolite concentrations were determined in hydrolyzed extracts of whole blood as well as of plasma. The 6TG concentration ranged from 3 to 18 nmol/ml blood in the 6TG group, in 7/10 samples 6TG was also detectable in plasma. The concentration however, was less than 3% compared to whole blood. In the 6MP group 6TG could not be detected in any of the analyzed plasma samples, at blood concentrations from about 1 to 2 nmol/ml. 6MeMP (as imidazole) was found in 3/10 hydrolysed plasma samples with levels not higher than 5% compared with those measured in whole blood (11–74 nmol/ml).

Table 1
Precision and accuracy of 6TG and 6MeMP in blood samples[a]

Compound	Concentration added (pmol/ml)	Concentration found (pmol/ml) (%)	CV (%)
Intra-assay ($n = 5$)			
6TG	20000	16704 (83.5)	6.2
	7500	5971 (79.6)	6.3
	2000	1646 (82.3)	6.5
	500	430 (85.6)	7.4
6MeMP	150000	147600 (98.4)	6.2
	50000	50350 (101)	9.6
	15000	16050 (107)	6.0
	3000	2991 (99.7)	10.6
Inter-assay ($n = 0$)			
6TG	0	3080	7.5
6MeMP	0	30736	9.1

[a] The accuracy was determined by adding known amounts of thioguanine (6TG) and 6-methylmercaptopurine (6MeMP) to whole blood. Five measurements were performed at every concentration. A pooled blood sample from patients under thiopurine treatment was used for inter-assay experiments.

3.5. Metabolite concentration in isolated RBC versus whole blood

In 15 samples (10 from 6MP-treated and 5 from 6TG-treated children) the metabolite concentrations were determined in isolated RBC and compared with the RBC related concentrations measured in whole blood (Table 2). The mean values/10^9 RBC were 6.5 (6MeMP) and 8% (6TG) higher in isolated cells than in whole blood. The differences were not significant in the MP-treated group (paired t-test, $P = 0.093$ for 6TG and 6MeMP), but were significant for 6TG ($P = 0.022$) if TG-treated patients were included in the calculation.

3.6. Patient samples

Table 3 shows the concentrations of 6TG and 6MeMP derivatives in six patients receiving an unchanged thiopurine dose for several weeks. The measurements ($n = 6$) in whole blood were performed 2 weeks after dose adjustment and then weekly or biweekly thereafter. In all these patients we found small intraindividual variations (CV < 20%) regarding the metabolite concentrations relative to the analyzed blood volume and also to the individual RBC count in this sample. The interindividual concentrations on the other side are very variable.

The metabolite concentrations at different time points during maintenance treatment in another patient receiving largely varying 6MP doses are depicted in Table 4. The measurements were done 3

Table 2
Concentrations of 6TG and 6MeMP derivatives in relation to the RBC count[a]

Sample	Drug	6TG derivatives (pmol/10^9 RBC)	6MeMP derivatives (pmol/10^9 RBC)
B1	6MP	1074	4674
E1		1215	4537
B2	6MP	842	61205
E2		913	72072
B3	6MP	492	7769
E3		439	8386
B4	6MP	420	17503
E4		488	18114
B5	6MP	328	11504
E5		403	12638
B6	6MP	263	16970
E6		233	17715
B7	6MP	437	33821
E7		498	36654
B8	6MP	333	5307
E8		358	5558
B9	6MP	361	67488
E9		413	78510
B10	6MP	423	19477
E10		380	18175
B11	6TG	1666	0
E11		1849	0
B12	6TG	1657	0
E12		1991	0
B13	6TG	2665	0
E13		3237	0
B14	6TG	4707	0
E14		4730	0
B15	6TG	2952	0
E15		3081	0

[a] The metabolite concentrations were determined in duplicate in whole blood (B) and in isolated RBC (E) of 10 patients under mercaptopurine (6MP) and of 5 patients under thioguanine (6TG) treatment. The mean values/10^9 RBC were 6.5% (6MeMP) and 8% (6TG) higher in isolated cells than in whole blood.

Table 3
Metabolite concentrations in the blood of patients receiving thioguanine or mercaptopurine during maintenance treatment[a]

Patient number	Drug	Dose (mg/m^2)	Dose ($\mu mol/m^2$)	6TG derivatives ($pmol/10^9$ RBC)	6TG derivatives (pmol/ml blood)	6MeMP derivatives ($pmol/10^9$ RBC)	6MeMP derivatives (pmol/ml blood)
1	6TG	14	83	790 ± 73 (9%)	3632 ± 340 (9%)	0	0
2	6TG	19	112	2939 ± 507 (15%)	11048 ± 1686 (15%)	0	0
3	6TG	35	211	3348 ± 532 (16%)	14061 ± 2019 (14%)	0	0
4	6MP	40	261	366 ± 56 (15%)	1447 ± 145 (10%)	9983 ± 1636 (16%)	36942 ± 6177 (17%)
5	6MP	75	493	194 ± 27 (14%)	595 ± 98 (16%)	54047 ± 9380 (14%)	165820 ± 30150 (18%)
6	6MP	97	636	407 ± 47 (12%)	1391 ± 178 (13%)	43569 ± 7322 (13%)	148703 ± 23535 (16%)

[a] The daily drug doses were unchanged for several weeks, the measurements in whole blood ($n = 6$) were performed weekly or biweekly beginning 2 weeks after dose adjustment. We found small intraindividual variations (CV < 20%) regarding the metabolite concentrations, the interindividual concentrations on the other side are very variable; values in parenthesis represent variation coefficient.

Table 4
Dose dependency of metabolite concentrations in a single patient receiving mercaptopurine during maintenance treatment[a]

Week	6MP dose (mg/m^2)	6TG derivatives ($pmol/10^9$ RBC)	6TG derivatives (pmol/ml blood)	6MeMP derivatives ($pmol/10^9$ RBC)	6MeMP derivatives (pmol/ml blood)
32	54	574	2382	1999	8296
47	107	769	2599	21402	72339
51	71	613	2176	7403	26281
54	54	553	2002	3171	11479
75	71	540	1739	5770	18579

[a] Measurements were performed in duplicate 3 weeks after dose adjustments. The concentration of 6TG derivatives raised about 1.5-fold, the concentration of derivatives with 6MeMP as base, on the other hand, raised about 10-fold in the given dose range of 6MP.

weeks after dose adjustments, the daily dose ranged from 54 to 107 mg/m^2. The concentrations of 6TG derivatives were between 540 and 769 $pmol/10^9$ RBC (1739–2599 pmol/ml blood), increasing about 1.5-fold. The concentration of derivatives with 6MeMP as base, on the other hand, raised about 10-fold, from 2 to 21 $nmol/10^9$ RBC (8–72 nmol/ml blood) in the given dose range of 6MP.

4. Discussion

There are a number of investigations dealing with the chromatographic determination of thiopurine metabolites in isolated erythrocytes. Extraction and deproteination can be performed easily and with good recoveries with perchloric acid [18,22]. As the determination of the nucleotides (mono-, di- and triphosphates) themselves requires time consuming gradient elution [23,24], mostly the bases liberated from the nucleotides by acidic [5,17–20,22,25] or enzymatic hydrolysis [26,27] are measured. The separation of RBC from whole blood, however, is laborious and requires several milliliters blood for convenient handling of the samples. Regular venipunctures, on the other hand, may be difficult especially in younger patients. It has never been shown before to our knowledge, that the isolation of RBC is necessary for measurements of thiopurine metabolites in these cells. The question is whether the concentration of these metabolites in plasma and/or WBC do indeed distort the determination of the concentration in RBC. Another investigation using whole blood for the determination of thiopurine metabolites was recently published by Pike et al. [28]. In this paper, we describe a new HPLC assay to determine 6TG and 6MeMP derivatives in very small blood samples collected from the finger-tip.

Nucleosides and nucleotides were extracted from whole blood with perchloric acid and hydrolysed to the corresponding base, sulfuric acid seems to be less efficient for this purpose [29]. 6MeMP is transformed under these conditions to 4-amino-5-(methylthio) carbonyl imidazole [21]. This may explain the very low recovery found in earlier investigations [17,19], in

contrast to our findings and those of Dervieux and Boulieu [21] measuring the imidazole instead 6MeMP itself. The formation of the imidazole is dependent on the pH [21]. As we have shown in this paper, a prolonged hydrolysis time at a somewhat higher pH, also results in complete transformation. The lower acid content used by us may be less aggressive to the HPLC equipment and to the reversed phase material. In contrast to previous investigations [5], we are using now a shorter and smaller column with the advantages of increased sensitivity, shorter analysis time, considerably reduced solvent consumption and reduced costs per analysis. The separation of 6TG and the imidazole can be performed in a single run under isocratic conditions and the samples can be injected every 10 min. With unsufficient concentrations of DTT as thiol protecting agent [30] we observed, however, even in blood of untreated patients an additional degradation product interfering with the imidazole peak. The limits of quantitation presented here are somewhat higher than those given by other groups [17,18,27]. The differences can be easily explained by larger sample and injection volumes or laborious extraction procedures described in previous investigations. The limits achievable with our new method, however, were sufficient for our purposes, more than 6000 patient samples were analyzed up to now.

Whereas Keuzenkamp [31] found no thiopurines in WBC after low doses of 6MP (25 mg/m^2), after intravenous and higher oral 6MP doses (more than 60 mg/m^2) derivatives of 6TG and 6MeMP are detectable in WBC [31,32]. TGN were also found in WBC of children taking 6TG, the range was similar to the range in children taking 6MP [33]. The concentrations, however, are too low to influence the determination of the metabolites in blood. Furthermore, the concentration of 6TG during 6TG treatment and that one of 6MeMP during 6MP treatment in hydrolysed plasma extracts was in every case less than 5% of the concentration in whole blood (if detectable at all). 6TG derivatives were not detectable in plasma of any patient treated with oral 6MP. Traces of 6TG riboside were found by Su et al. [34] during a high dose iv 6MP infusion and also after oral 6TG uptake [5]. We did not investigate whether or not these bases were liberated during hydrolysis from metabolites. 6TG [35–37] and also 6MeMP [31], however, are eliminated very rapidly from plasma; the concentration of 6MeMP riboside, on the other hand, remains constant for several hours after MP infusion [31]. Thus, we assume that the small amounts of bases in hydrolyzed plasma samples may be derived mainly from metabolites.

As shown in this paper and also by Pike et al. [28], 6TG and 6MeMP values measured in whole blood were closely coincident with those obtained from isolated RBC, when related to the RBC count. This observation also demonstrates that the determinations of erythrocytic 6TG and 6MeMP derivatives in whole blood were not markedly influenced by metabolites in plasma or white blood cells. In other words, the bases measured after hydrolysis were liberated nearby completely from the metabolites (nucleotides) accumulated in RBC. From our results we therefore conclude that the isolation of RBC is not necessary for measurements of thioguanine and mercaptopurine metabolites in these cells, if the drugs were given per os in the day before blood sampling as was the case in our patients. Azathioprine has been used for many years both after organ transplantation and for auto-immune diseases. This thiopurine is metabolized in the similar manner as 6MP. We have, however, not proved extensively whether the limits of quantitation of our method are sufficient to determine these metabolites in capillary blood. Whether this method is suitable for such determinations after intravenous drug administration remains also to be investigated.

The interindividual erythrocytic metabolite concentration is highly variable [5,11,14,17,38]. This may depend on the individual drug resorption and/or activities of the enzymes involved in the intracellular thiopurine metabolism. If the drug doses were unchanged for several weeks, we found only small intraindividual variations in hydrolysed blood extracts regarding the 6TG concentrations in the 6TG as well as the 6TG and 6MeMP concentrations in the 6MP branch. Similar observations were made for erythrocytic TGN during 6MP [11,39] and azathioprine treatment [40,41]. The concentrations of TGN during unchanged 6TG and of 6MeMP nucleotides during unchanged 6MP doses have never been investigated before to our knowledge. Decreases or larger intraindividual variations of the concentration of the thiopurine metabolites at unchanged doses were often an indicator of noncompliance, other reasons such as diarrhea, however, may also result in lower metabolite concentrations in some cases (in preparation, [16]).

As we have shown in this paper, the concentration of 6MeMP nucleotides in a single patient is much more dependent on the given 6MP dose than the concentration of TGN. Thus, measurements of the methylated metabolites may be of a greater value to assess the individual compliance than measurements of TGN.

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References

[1] G. Elion, Science 224 (1989) 41.
[2] G. Janka-Schaub, N. Erb, D. Harms, Med. Pediatr. Oncol. 23 (1994) 197.
[3] G. Janka-Schaub, D. Harms, N. Erb, H. Spaar, U. Graupner, U. Goebel, N. Jorch, K. Winkler, Med. Pediatr. Oncol. 27 (1996) 213.
[4] J. Aarbakke, G. Janka-Schaub, G. Elion, Trends Pharmacol. Sci. 188 (1997) 3.
[5] N. Erb, D. Harms, G. Janka-Schaub, Cancer Chemother. Pharmacol. 42 (1998) 266.
[6] J.A. Nelson, J.W. Carpenter, L.M. Rose, D.J. Adamson, Cancer Res. 35 (1975) 2872.
[7] D.M. Tidd, A.R. Paterson, Cancer Res. 34 (1974) 738.
[8] J.P.M. Bökkerink, E.H. Stet, R.A. De Abreu, F.J.M. Damen, T.W. Hulscher, M.A.H. Bakker, J.A. van Baal, Biochem. Pharmacol. 45 (1993) 1455.
[9] T. Dervieux, J.G. Blanco, E.Y. Krynetski, E.F. Vanin, M.F. Roussel, M.V. Relling, Cancer Res. 61 (2001) 5810.
[10] L. Lennard, C.A. Rees, J.S. Lilleyman, J.L. Maddocks, Br. J. Clin. Pharmacol. 16 (1983) 359.
[11] K. Schmiegelow, I. Bruunshuus, Cancer Chemother. Pharmacol. 26 (1990) 288.
[12] D.L. Lancaster, L. Lennard, K. Rowland, A.J. Vora, J.S. Lilleyman, Br. J. Haematol. 102 (1998) 439.
[13] B. Bostrom, G. Erdmann, Am. J. Pediatr. Hematol. Oncol. 15 (1993) 80.
[14] J.S. Lilleyman, L. Lennard, Lancet 383 (1994) 1188.
[15] L. Lennard, J. Welch, J.S. Lilleyman, Br. J. Cancer 72 (1995) 1004.
[16] D.O. Harms, N. Erb, R. van de Loo, G. Janka-Schaub, Blood 96 (2000) 721a.
[17] L. Lennard, H.J. Singleton, J. Chromatogr. 583 (1992) 83.
[18] T. Dervieux, R. Boulieu, Clin. Chem. 44 (1998) 551.
[19] G.R. Erdmann, L.A. France, B.C. Bostrom, D.M. Canafax, Biomed. Chromatogr. 4 (1990) 47.
[20] K. Rowland, L. Lennard, J.S. Lilleyman, J. Chromatogr. B 705 (1998) 29.
[21] T. Dervieux, R. Boulieu, Clin. Chem. 44 (1998) 2511.
[22] R. Boulieu, A. Lenoir, C. Bory, J. Chromatogr. 615 (1993) 352.
[23] L.E. Lavi, J.S. Holcenberg, Anal. Biochem. 144 (1985) 514.
[24] C.W. Keuzenkamp, R.A. De Abreu, J.P. Bökkerink, J.M. Trijbels, J. Chromatogr. B 672 (1995) 53.
[25] I. Bruunshuus, K. Schmiegelow, Scand. J. Clin. Lab. Invest. 49 (1989) 779.
[26] E.Y. Krynetski, N.F. Krynetskaia, Y. Yanishevski, W.E. Evans, Mol. Pharmacol. 47 (1995) 1141.
[27] T. Giverhaug, S. Bergan, T. Loennechen, H. Rugstad, J. Aarbakke, Ther. Drug Monit. 19 (1998) 663.
[28] G.M. Pike, C.L. Franklin, D.C. Mays, J.L. Lipsky, P.W. Lowry, W.J. Sandborn, J. Chromatogr. B 757 (2001) 1.
[29] M. Shipkova, V.M. Armstrong, E. Wieland, M. Oellerich, Clin. Chem. 49 (2003) 260.
[30] W.W. Cleland, Biochemistry 3 (1964) 480.
[31] C.W. Keuzenkamp, Thesis, Nijmegen, 1996, p. 109.
[32] T. Dervieux, Y. Chu, Y. Su, C.-H. Pui, W.E. Evans, M.V. Relling, Clin. Chem. 48 (2002) 61.
[33] D.L. Lancaster, N. Patel, L. Lennard, J.S. Lilleyman, Cancer Chemother. Pharmacol. 50 (2002) 33.
[34] Y. Su, Y.Y. Hon, Y. Chu, M.E.E. van den Poll, M.V. Relling, J. Chromatogr. B 732 (1999) 459.
[35] L.W. Brox, L. Birkett, A. Belch, Cancer Chemother. Pharmacol. 6 (1981) 35.
[36] L. Lennard, H.A. Davies, J.S. Lilleyman, Br. J. Cancer 68 (1993) 186.
[37] J. Lilliemark, B. Pettersson, M. Järnmark, C. Peterson, Leuk. Lymphoma 4 (1991) 271.
[38] T. Dervieux, Y. Médard, P. Verpillat, V. Guigonis, M. Duval, B. Lescoeur, S. Suciu, E. Vilmer, E. Jacqz-Agrain, Leukemia (2001) 15.
[39] L. Lennard, J.C. Welch, J.S. Lilleyman, Br. J. Clin. Pharmacol. 44 (1997) 455.
[40] S. Bergan, H.E. Rugstad, Ø. Bentdal, L. Endresen, O. Stokke, Ther. Drug Monit. 16 (1994) 13.
[41] G. Chan, G. Erdmann, S. Gruber, A. Matas, D. Canafax, J. Clin. Pharmacol. 30 (1990) 358.