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High-performance liquid chromatographic assay for the measurement of azathioprine in human serum samples

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Abstract

A quantitative, highly sensitive HPLC-based method for the direct measurement of azathioprine is described, introducing a newly synthesized 9-methyl derivative of this immunosuppressant as internal standard in combination with isocratic HPLC and UV-absorbance measurement at 285 nm. Analysis was performed on a RP18 select B column with acetonitrile–0.01 *M* potassium phosphate buffer (12:88, v/v) at pH 2.3 as mobile phase. Results of precision analysis from serum samples spiked with 3.125, 12.5, and 25 ng azathioprine, respectively, were (mean±S.D.): 3.148±0.259 ng (8.22%), 12.594±0.571 ng (4.53%), and 25.016±0.658 ng (2.63%) with C.V. values in parentheses for $n=5$. The accuracy of the assay ranged from −7.6 to 0.7% (expressed as % bias) tested on five consecutive days. The limit of quantification was at 2.5±0.256 ng (C.V. 10.25%), thus allowing drug monitoring in long-term patients. The method can also be used to evaluate individual pharmacokinetic parameters of a single patient, as well as for drug monitoring of a cohort of patients who suffer from azathioprine-induced symptoms of toxicity. An example of the pharmacokinetic behaviour in an individual is given in this paper.

Keywords: Azathioprine

1. Introduction

Azathioprine [6-(1-methyl-4-nitroimidazol-5-ylthio)purine] is an immunosuppressant agent which has been clinically used for the past thirty years in the prevention of organ rejection [1–5] and in the management of several autoimmunological disorders (e.g. rheumatoid arthritis, colitis ulcerosa, autoimmunological hepatitis) [6–9]. Its metabolism has been studied extensively [10–15]. In humans, it is mainly a non-enzymatic nucleophilic attack that converts azathioprine to the active metabolite 6-mercaptopurine (6-MP), which acts as an antimetabolite to DNA and RNA synthesis (Fig. 1). This results in a mild antiproliferative effect of azathioprine aimed at the proliferation of lymphocytes. Therefore, azathioprine is considered to be a prodrug of 6-mercaptopurine. In vitro studies provide evidence that azathioprine also acts directly on DNA synthesis. So far, it is unknown to what extent azathioprine or its major metabolite 6-mercaptopurine contribute to the immunosuppressant effect and toxicity [11,16]. Azathioprine is orally applied in doses from 0.5 to 3 mg/kg body weight. Intravenous injection is avoided in patients because of unwanted side effects and is of no practical use for long-term

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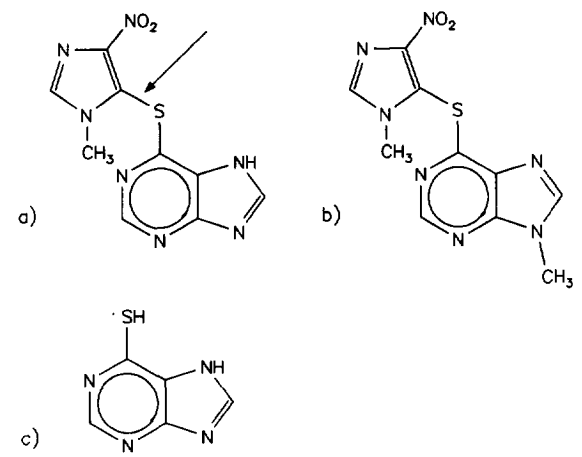

Fig. 1. Structural formulas of azathioprine, 6-mercaptopurine and 9-methylazathioprine. (a) Azathioprine [6-(1-methyl-4-nitroimidazol-5-ylthio)purine] is rapidly converted to (c) 6-mercaptopurine by non-enzymatic, nucleophilic attack (↓) and successive cleavage of its internal thioether-bond. (b) A 9-methyl derivative of azathioprine [9-methyl-6-(1-methyl-4-nitroimidazol-5-ylthio)purine] was synthesized as an internal standard for quantitative HPLC-analysis of serum samples.

treatment. Some patients experience unpredictable acute myelosuppression, consecutive leucopenia and hepatotoxicity [17,18], often necessitating dose reduction or even cessation of therapy. It is not yet clear whether the toxic effect of azathioprine is a consequence of the pharmacokinetics of the drug (e.g. enhanced bioavailability, which might result in significantly higher plasma levels in affected individuals). Therefore we have developed a simple, highly sensitive HPLC-based method for the direct measurement of azathioprine levels in plasma samples, allowing studies on steady state concentrations in oral, long-term azathioprine therapy and evaluation of the drug's pharmacokinetics in individuals.

Azathioprine (M_r 277.3, pK_a 8.2, Fig. 1a) is a pale yellow powder, insoluble in water and poorly soluble in ethanol and chloroform, and moderately soluble in acetonitrile and in dilute solutions of alkali hydroxides, although the latter is associated with some decomposition. Its melting point is about 238°C at which temperature it decomposes, thus evading analysis by gas chromatography. Previously published methods employing HPLC are based on the detection of its 6-MP metabolite (Fig. 1c), which can be more easily extracted from plasma than azathioprine and detected by its UV absorbance [13,19–22]. Methods aiming at the detection of azathioprine itself are not sensitive enough as to record the low plasma levels (<10 ng/ml) expected during oral steady-state treatment [21,23]. To achieve satisfactory precision in quantification in this low concentration range, the internal standard (I.S.) method was employed. Lin et al. [23] used benzazimide as I.S., which was unavailable to us. We therefore synthesized a new compound serving as an I.S. To qualify for use as an I.S., a compound would have to be more lipophilic than azathioprine —resulting in an extended retention time— while the extraction behaviour would have to resemble that of azathioprine to yield constant ratios of peak areas over a broad concentration range. A compound meeting these requirements was a 9-methyl derivative of azathioprine (9-M-Aza), which was obtained by methylation of the parent substance with methyliodide in absolute dimethylformamide in the presence of potassium carbonate. The N-methylation of azathioprine in the 9-position to 9-M-Aza (Fig. 1b) was demonstrated by spectroscopic and molecular data. The melting point of 174±5°C distinguishes it from the previously synthesized 7-methyl derivative with a melting point of 254±5°C [26].

2. Experimental

2.1. Reagents

Azathioprine and Imurek were provided by Wellcome (Burgwedel, Germany). All solvents were HPLC-grade from Merck (Darmstadt, Germany). Water for eluent preparation was from a Millipore purification system. Methyliodide and absolute dimethylformamide were obtained from Fluka (Neu-Ulm, Germany).

2.2. Instrumentation and chromatographic conditions

The HPLC system used consisted of a Model 480 high-precision pump, a degassing device, an electronically controlled column oven, and a photodiode-array UV detector (UVD 320) equipped with a 12-μl

flow cell, all from Gynkotek (Munich, Germany). Both Aza and 9-M-Aza were measured at a wavelength of 285 nm (Fig. 2). Data were sampled and analyzed on a Gynkotek C-R5A integrator. For acquisition of spectral data and peak-purity trials the UVD 320 detector was used in combination with a microcomputer-based analysis program (Gynkosoft V5.21). Chromatographic separation was performed at 22°C on a Merck LiChrospher 60 RP-select B column (250×4.6 mm, particle size 5 μm), protected by a Merck LiChrospher 100 RP 18 precolumn

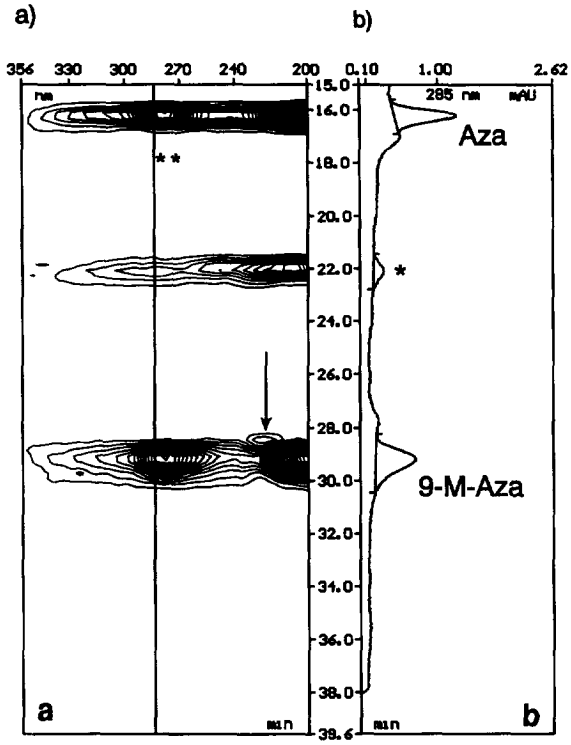

Fig. 2. (a) Contour plot and (b) chromatogram of serum sample containing 25 ng/ml of Aza and 9-M-Aza. (a) Contour plot of absorbance scans from 360 to 200 nm of a serum sample spiked with 25 ng/ml of azathioprine (Aza) and 9-methylazathioprine (9-M-Aza); ** indicates the detection wavelength of 285 nm; (↓) shows an unknown serum component with a peak absorbance at ca. 240 nm which would substantially interfere with the detection of 9-methylazathioprine at 220 nm, since both substances are not well resolved under these chromatographic conditions. (b) Absorbance chromatogram of the same run at 280 nm. Besides Aza and 9-M-Aza, an unknown serum component could be detected at this wavelength (*). Superior chromatographic resolution allows it to be clearly separated from both peaks of interest.

(4×4.6 mm, particle size 5 μm). The mobile phase consisted of 12% acetonitrile and 88% 0.01 *M* potassium phosphate buffer (v/v), pH 2.3, and was passed through a disposable filter (0.2-μm pore width) prior to use. After each run with a plasma sample the column was flushed for 2 min with acetonitrile–buffer (1:1) to wash out contaminating substances with long retention times. The flow-rate was 1000 μl/min with t_0=1.9 min. The injection volume was 0.2 ml. Under these conditions the number of theoretical plates was approximately 12 000 for calibration standards and approximately 8000 for serum samples. The precolumn was changed every 100 serum sample runs and the column was replaced when the plate number had decreased below 6000.

2.3. Synthesis of 9-methyl azathioprine (9-M-Aza)

A-400 mg (2.90 mmol) quantity of dried potassium carbonate and 0.2 ml (3.2 mmol) of methyliodide were added to a solution of 220 mg (0.79 mmol) of 6-(1-methyl-4-nitroimidazol-5-ylthio)-purine in 7 ml absolute dimethylformamide at a temperature of 0–5°C. After stirring in a nitrogen atmosphere for 24 h at room temperature, the cloudy reaction mixture was diluted with 14 ml of demineralized water. The product was precipitated by neutralization with 1 *M* hydrochloric acid and 1 *M* sodium hydrogencarbonate solution, collected by suction, washed with water and dried in a vacuum yielding 99.4 mg (44%) of 9-methyl-6-(1-methyl-4-nitroimidazol-5-ylthio)purine (9-M-Aza; yield not optimized) with a melting point at 171±2°C. Thin-layer chromatography (TLC) on silica gel 60 F 254 (Merck) in acetone; Aza: R_F=0.24; 9-M-Aza: R_F= 0.35. Analytically pure 9-M-Aza with the melting point of 174–175°C was obtained by repeating the precipitation from dimethylformamide solution with water.

IR (KBr): 3400, 3120, 3103, 3070, 1587, 1566, 1533, 1500, 1377, 1333, 1301, 1242, 1220, 933, 833, 641 cm^{-1}.

^{1}H-NMR (DMSO-$^2H_6$)=3.45 (s, H_2O); 3.74 (s, CH_3); 3.86 (s, CH_3); 8.29 (s, CH); 8.55 (s, CH); 8.64 (s, CH) ppm.

^{13}C-NMR (DMSO-$^2H_6$)=29.91 (CH_3); 33.08

(CH_3); 117.20; 130.44; 139.51 (CH); 146.82 (CH); 149.94; 150.22; 151.59 (CH); 155.14 ppm.

MS (150°C) m/e=291 (0.03; M^+), 275 (5), 261 (13), 246 (17), 245 (100), 230 (8), 204 (6), 177 (11), 160 (15), 150 (13), 133 (64), 106 (15), 79 (18).

Anal. cal. for $C_{10}H_9N_7O_2 \cdot H_2O$: C 38.84; H 3.59; N 31.71; found: C 38.92; H 3.64; N 31.65.

All concentrations for 9-M-Aza given in this paper are expressed as its monohydrate.

2.4. Preparations of standards for amount/response calibration

Stock solution was 5% dimethylsulfoxide (DMSO) in acetonitrile containing caffeine (1 mg/ml), azathioprine (1 mg/ml) and 9-methylazathioprine (1 mg/ml). Caffeine was used as a hydrophilic component of the calibration standard mixture to monitor elution of substances with short retention times. Calibration standards were prepared by diluting the stock solution (625 ng/ml of each substance) with an appropriate amount of the eluent. Stored at room temperature, they were stable for four weeks before they were prepared afresh.

2.5. Serum sample preparation

Serum was spiked by addition of appropriate amounts of stock solution to the desired serum concentrations of azathioprine and 9-methylazathioprine, respectively, in screw-capped polyethylene tubes. Prior to further processing, samples remained at room temperature for 30 min to allow equilibration between free and serum-bound portions of the compounds before further processing was initiated.

Blood samples were centrifuged immediately after collection. Following coagulation of the plasma fraction the serum was removed and centrifuged once to remove coagulated protein clots.

Two 0.5-ml volumes of serum were transferred into two polyethylene tubes and 4.5 ml of ethylacetate were added to each. After vigorous shaking (vortex) for 1 min, followed by centrifugation (1 min), the solvent phase were combined in a glass tube. This extraction was repeated once. The combined solvent phases were vacuum-dried at 35°C and the residues were reconstituted with 0.25 ml of eluent by vortex-mixing for 10 s, followed by 10 min sonification prior to injection.

2.6. Calibration and recovery of azathioprine from serum samples

An aliquot of 1 ml serum was spiked with different concentrations of Aza (3.125 to 50 ng/ml, five samples per concentration) and 50 ng/ml of 9-M-Aza. Peak areas for Aza and 9-M-Aza were related to the amount/response ratios in chromatograms obtained from calibration standards. For evaluation of accuracy and precision of the assay three 5-ml serum aliquots were spiked with 3.125 ng, 12.5 ng, and 25 ng of azathioprine, respectively, and analyzed on five consecutive days.

2.7. Stability of azathioprine and 9-methylazathioprine in blood

The thioether bond within the azathioprine molecule is cleaved by non-enzymatic nucleophilic attack (e.g. by glutathione) in the liver and during contact with erythrocytes. No data exist for 9-methylazathioprine, but its structural similarity to azathioprine, especially in the region of the sulphur bridge, suggests a similar breakdown for this derivative. To examine whether azathioprine and 9-methylazathioprine concentrations decrease during prolonged contact with human blood, a 50-ml fresh blood sample was spiked with 25 ng/ml of each substance and gently agitated at 37°C. Test samples of 5 ml were drawn after 5, 15, 30, 60, 90, and 120 min and processed as described.

3. Results

3.1. Chromatographic data for azathioprine and 9-methylazathioprine

Both substances were eluted from the column with sufficient resolution. Aza, which is more hydrophilic than 9-M-Aza had a shorter retention time than 9-M-Aza. The k' values were 6.20±0.12 for Aza and 11.53±0.20 for 9-M-Aza as obtained from twelve consecutive runs of spiked serum samples. The delayed elution of 9-methylazathioprine resulted in a

substantial broadening of the peak, but this did not interfere with its correct integration. The main metabolite of Aza is 6-mercaptopurine (6-MP). This substance was not detectable at the used wavelength, because it has an absorbance maximum at 330 nm. Due to its short retention time (4.5 min) 6-MP was concealed by high-absorbance background induced by serum components which could not sufficiently be separated from the injection sample without decreasing the recovery of Aza.

3.2. Spectral data of azathioprine and 9-methylazathioprine

Serum samples were spiked with 25 ng/ml of Aza and 9-M-Aza. Spectra covering the range from 220 to 400 nm were continuously scanned from 15 to 40 min. Both substances had one peak absorbance below 220 nm and another peak absorbance at 285 nm, the latter appearing the most suitable for detection (Fig. 2). At that wavelength no disturbance of the absorbance signal by contaminating serum constituents could be observed. Peak-purity trials performed at 285 nm gave results >95% for both Aza and 9-M-Aza.

3.3. Amount/response calibration

Suitable volumes of calibration standards were chosen so as to allow Aza and 9-M-Aza injections in the range from 3.125 ng to 50 ng onto the column. The peak areas (PA) of responses for both substances were determined, and a linear regression was performed (for results see Table 1). Best results were obtained by weighted fitting of the peak areas to $1/(\text{amount})^2$. The coefficient of variation (C.V.) for absolute peak areas was below 4.0% throughout all concentrations of both, Aza and 9-M-Aza.

3.4. Calibration and quantitative analysis of spiked serum samples

Drug-free serum aliquots were spiked with the indicated concentrations of Aza and 50 ng/ml of 9-M-Aza. Representative chromatograms of processed serum samples are depicted in Fig. 3b. Peak areas (PA) for both substances were determined and peak ratios (Aza/M-Aza=Q) were derived and the resulting calibration curve was analyzed for linearity yielding:

$$\text{Aza (ng/ml)} = \frac{Q}{(1.815 \cdot 10^{-2} \pm 1.785 \cdot 10^{-4})} + (1.628 \cdot 10^{-2} \pm 5.904 \cdot 10^{-3})$$

Means±standard errors were derived from regression analysis of serum spiked with 3.125, 6.25, 12.5, 18.75, 25, and 50 ng azathioprine/ml with five independent samples identically processed for each concentration Table 2. The accuracy and precision was tested by analyzing serum samples containing 3.125 ng, 12.5 ng, and 25 ng of azathioprine with 50 ng of 9-M-Aza added to each sample on five consecutive days. Results were (mean±S.D.): 3.148±0.259 ng (8.22%), 12.594±0.571 ng (4.53%), and 25.016±0.658 ng (2.63%) with C.V. values in parentheses. The limit of quantification (LOQ) was determined as a concentration of Aza, at which the C.V. values exceeded 10% and computed at 2.5 ng Aza/ml.

3.5. Stability

Measured concentrations of Aza and 9-M-Aza were fitted by non-linear regression to a first-order exponential function (Fig. 4 and Table 3). The obtained k values were converted to half-life times $t_{1/2}$ for both substances. In serum samples stored at

Table 1
Parameters of linear regression from amount/response chromatograms

Compound	Slope (mean±S.D.) (mAU s ng^{-1})	Intercept (mean±S.D.) (mAU s)	r^2
Azathioprine	4.338±0.06988	0.8839±0.4352	0.99968
9-Methyl-azathioprine	4.053±0.09799	1.252±0.6245	0.99989

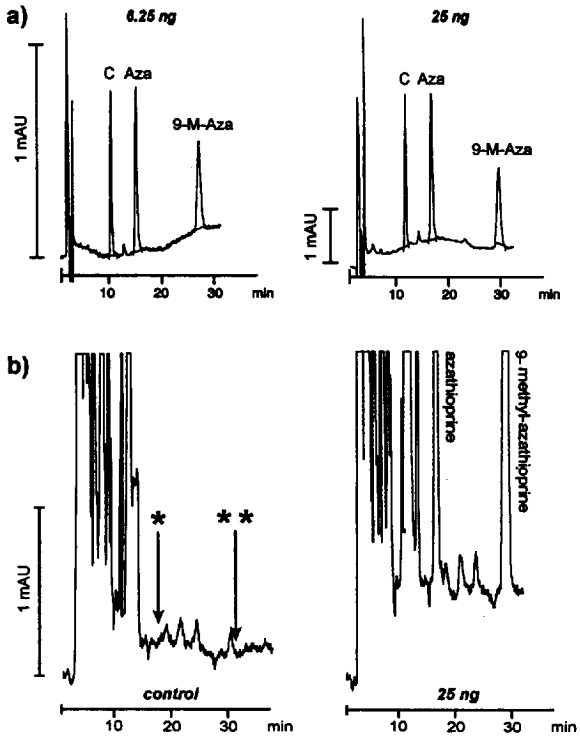

Fig. 3. Chromatograms of (a) calibration runs and (b) serum samples. (a) Suitable amounts of calibration standards were used to achieve concentrations of caffeine (C), azathioprine (Aza) and 9-methylazathioprine (9-M-Aza), respectively, as indicated. For chromatographic conditions refer to text. Note the different calibration of the ordinate; increased sensitivity of detection (figure on the left) leads to enhancement of baseline signal noise. (b) Plain (control) and spiked serum samples were processed as described in the text and analyzed by HPLC. In the resulting control chromatogram, * and ** indicate retention times of Aza and 9-M-Aza.

−20°C no significant decrease in the concentrations of Aza and 9-M-Aza could be detected for up to four weeks.

Table 2
Recovery of azathioprine from serum samples

Concentration (ng/ml)	Azathioprine		9-Methylazathioprine	
	Mean (%)	C.V. (%)	Mean (%)	C.V. (%)
6.25	39.54	9.88	n.d.	
12.5	41.88	10.38	n.d	
25	55.70	4.10	n.d	
50	63.57	9.9	75.25	11.26

n=5 for each concentration.

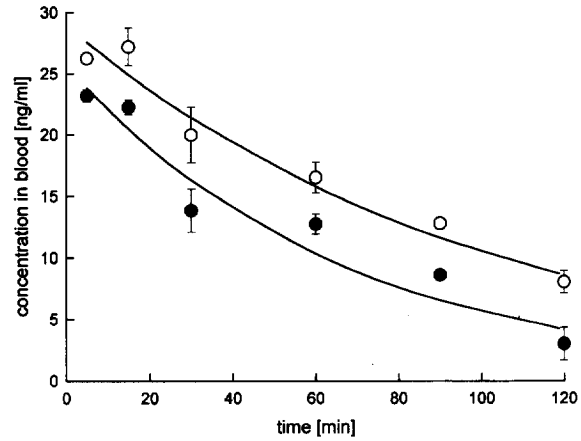

Fig. 4. Time course of concentration decrease of (○) Aza and (●) 9-M-Aza during prolonged contact with blood. For experimental procedures and curve-fitting results please refer to text. Values are means of three independent analysis procedures; error bars indicate S.D.

3.6. Individual pharmacokinetic parameters

A 30-year old, healthy, male volunteer (body weight 82 kg) ingested 150 mg of azathioprine (three tablets of Imurek) at t=0. Serum samples were drawn at times indicated in Fig. 5 and processed as described. Serum concentrations increased from 15 to 45 min reaching a maximum of ca. 33 ng/ml. This invasion was followed by a decline of azathioprine concentration to ca. 13 ng/ml after 120 min. The volume of distribution V_D, the fraction of resorption f and the time constants for invasion k_a and evasion k_e from the blood compartment were determined by fitting the time course of serum concentrations to the following model:

$$C_t = \frac{fD}{V_D}\left(\frac{k_a}{k_a - k_e}\right)(e^{-k_e t} - e^{k_a t})$$

with t in min. Parameters estimated by nonlinear regression were: D=150 mg, f=12.8%, V_D=1.8 l/kg, k_a=0.029 and k_e=0.03. These data agree well with pharmacokinetic data obtained from several other sources [13,22,24]. Comparative studies using the i.v. administration route were not performed at this point of time due to the substantial unwanted effects accompanied by i.v. application of the drug.

Table 3
Parameters of exponential decrease of azathioprine and 9-methylazathioprine during prolonged contact with blood

Compound	C_0 (mean±S.D.) (ng/ml)	k (mean±S.D.) (min^{-1})	$t_{1/2}$ (min)
Azathioprine	29.03±1.638	$10.16 \cdot 10^{-3} \pm 0.83 \cdot 10^{-3}$	68
9-Methylazathioprine	25.70±2.787	$15.18 \cdot 10^{-3} \pm 2.34 \cdot 10^{-3}$	45

$C(t)=C_0 \cdot e^{-kt}$.
$n=3$ individually processed samples.

4. Discussion

The HPLC method presented here is sufficiently sensitive to quantitate azathioprine concentrations with an LOQ of 3 ng/ml, allowing drug monitoring in long-term oral treatment with this substance. The newly synthesized 9-methyl derivative of azathioprine appeared to be a compound suitable as internal standard, since it has a linear absorption response and an extraction behaviour similar to that of azathioprine. Both compounds have an absorbance maximum at 285 nm which allows the described technique also to be used with single wavelength detection. Although there is an even higher absorbance at 220 nm we were concerned about high background absorbance at this wavelength caused by coextracted serum components. To set the detection wavelength for both substances to 285 nm therefore represents a compromise with respect to sensitivity and specificity. The extraction procedure itself is rapid and avoids complex steps or toxic reagents in sample preparation. It is desirable to improve the method for simultaneous monitoring of the serum concentration of 6-MP, because it is the main metabolite of Aza. The short retention time of 6-MP in this assay makes it difficult to clearly separate it from serum constituents having similar UV-absorbances at 330 nm. Performing less sensitive preparation methods to clear contaminating substances from the sample has led to substantial loss of Aza and a decrease of recovery in preliminary experiments.

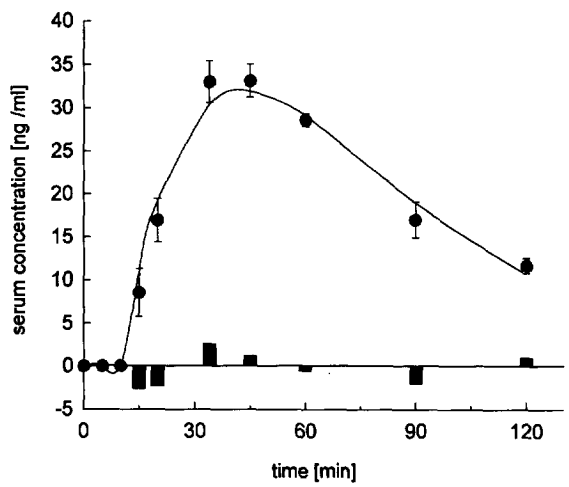

Fig. 5. Serum concentration of azathioprine after oral application. Experimental details are given in the text. Symbols represent the mean of three individually processed serum samples at indicated times (abscissa), error bars denote S.D. The bars show the residuals after fitting the raw data to the model described in the text.

Studies on the stability of Aza and 9-M-Aza indicate that the serum has to be separated from blood samples as early as possible to prevent degradation of azathioprine. This fact has also been emphasized by several other groups of researchers [10,11,25]. Similarly, 9-methylazathioprine undergoes a putative non-enzymatic cleavage which appears even to be accelerated. This may be due to the facilitated entry of this more lipophilic substance into erythrocytes. If processed, however, serum samples could be stored for at least four weeks with no significant decline of either Aza or 9-M-Aza concentrations.

Unexpectedly the absorbed fraction of orally applied azathioprine was as low as 12.8%. Increased absorption would surely result in significantly higher serum concentrations of azathioprine, which may be accompanied by toxic effects of the drug. At least two independent processes contribute to the decline of azathioprine blood levels. The first one is the

passage of the substance from the intravascular space into the tissues and cells. The second mechanism that is involved in the decrease of azathioprine blood levels is the fast degradation of the substance by reductive cleavage of the thioether-bond. This was demonstrated by incubating spiked blood samples and measuring declining azathioprine concentrations. In future studies serum samples drawn from azathioprine-treated patients should be analyzed and untoward effects monitored to detect a possible correlation between abnormal serum concentrations of azathioprine and signs of toxicity. Azathioprine is sometimes used in combination with other immunosuppressant drugs (e.g. glucocorticoids or cyclosporin A). Analysis of samples drawn from patients treated with combination therapies gave no evidence for any interference of these coadministered substances with the presented assay. The study of pharmacokinetics in a sufficient number of azathioprine-treated individuals may allow insight into the underlying parameters and reveal how changes in these could account for alteration in the drug's properties and effects in man.

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References

[1] S.M. Lim and D.J. White, Ann. Acad. Med. Singapore, 20 (1991) 144.
[2] A.I. Lazarovits, Clin. Invest. Med., 12 (1989) 311.
[3] D.F. du Toit and J.J. Heydenrych, S. Afr. Med. J., 70 (1986) 687.
[4] G.L. Chan, D.M. Canafax and C.A. Johnson, Pharmacotherapy, 7 (1987) 165.
[5] G.B. Elion and G.H. Hitchings, Handbook of Experimental Pharmacology, Vol. XXXVIII/2, Springer Verlag, Berlin, 1975.
[6] D.A. Fox and W.J. McCune, Concepts Immunopathol., 7 (1989) 20.
[7] T.J. Counihan and C. Feighery, Ir. J. Med. Sci., 160 (1991) 199.
[8] V.C. Ho and D.M. Zloty, Dermatol. Clin., 11 (1993) 73.
[9] D.J. Nashel, Med. Clin. North Am., 69 (1985) 817.
[10] K.G. Van Scoik, C.A. Johnson and W.R. Porter, Drug Metab. Rev., 16 (1985) 157.
[11] L. Lennard, Eur. J. Clin. Pharmacol., 43 (1992) 329.
[12] G.L.C. Chan, G.R. Erdmann, S.A. Gruber, P. Stock, S. Chen, N.L. Ascher and D.M. Canafax, Eur. J. Clin. Pharmacol., 36 (1989) 265.
[13] B. Odlind, P. Hartvig, B. Lindström, G. Lönnerholm, G. Tufveson and N. Grefberg, Int. J. Immunopharmacol., 8(1) (1986) 1.
[14] K.G. v. Scoik, C.A. Johnson and W.R. Porter, Drug Metab. Rev., 16 (1985) 157.
[15] K.G. v. Scoik and C.A. Johnson, Drug Metab. Rev., 16 (1 and 2) (1985) 157.
[16] C.E. Voogd, Mutat. Res., 221 (1989) 133.
[17] S.J. Rossi, T.J. Schroeder, S. Hariharan and M.R. First, Drug Safety, 9 (1993) 104.
[18] D.I. Min and A.P. Monaco, Pharmacotherapy, 11 (1991) 119S.
[19] V. Kurowski and H. Iven, Cancer Chemother. Pharmacol., 28 (1991) 7.
[20] H.J. Wypior, A. Schilling, L. v. Meyer and W. Land, Res. Exp. Med. (Ber.), 181 (1882) 21.
[21] J.L. Maddocks, Br. J. Clin. Pharmacol., 8 (1979) 273.
[22] T.L. Ding and L.Z. Benet, Drug Metab. Dispos., 7(6) (1979) 373.
[23] S. Lin, K. Jessup, M. Floyd, T.-P.F. Wang, T. v. Buren, R.M. Caprioli and B.D. Kahan, Transplatation, 29 (1980) 290.
[24] R. Venkataramanan, K. Habucky, G.J. Burckart and R.J. Ptachcinski, Clin. Pharmacokin., 16 (1989) 134.
[25] L. Lennard and J.L. Maddocks, J. Pharm. Pharmacol., 35 (1983) 15.
[26] U.S.S.R. patent 384822 by P.M. Kochergin, V.S. Korsunskii and V.S. Shlikhunova, 1973.